THE EFFECTS OF FEEDING HIGH-LINOLEIC SAFFLOWER SEEDS ON ESTRUS SYNCHRONIZATION IN BEEF HEIFERS

by

Kaitlyn Claire Spence

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Animal and Range Sciences

MONTANA STATE UNIVERSITY
Bozeman, Montana

July 2014

ACKNOWLEDGEMENTS

I would like thank my wonderful family for their love and support through my whole college career. I would especially like to thank my parents, Dennis and Susan Spence, for sharing their wisdom and inspiring my passion and love for all animals great and small.

Thank you to my major professor, Dr. Glenn Duff, for giving me the opportunity to further my education. I would also like to thank my co-advisor, Dr. Jan Bowman, for her time and endless support during the pursuit of this degree. I would like to thank my committee members, Jim Berardinelli, Darrin Boss, Tom Geary, and Paul Grieco for their assistance and support throughout the course of my graduate training.

Thank you to the staff at both Bozeman Agricultural Research and Teaching facility and Northern Agricultural Research Center (NARC) for all their help. I would especially like to thank Julia Dafoe for her friendship and help at the NARC. I would also like to thank my Montana family, the Jakob family, for always believing in me. Finally, a huge thank you to all the fellow graduate and undergraduate students for their generous support throughout this project.

TABLE OF CONTENTS

TABLE OF CONTENTS - CONTINUED

LIST OF TABLES

Table Page

LIST OF TABLES – CONTINUED

Table Page

LIST OF FIGURES

Figure Page

ABSTRACT

The objectives of these studies were to investigate the effects of feeding high-linoleic cracked safflower seeds on reproductive and performance outcomes in pubertal beef heifers. Three experiments were conducted at Montana State University research facilities (Exp. 1, n = 48, BW = 370 ± 24.8 kg; Exp. 2, n = 40, BW = 396.5 ± 31.7 kg; Exp. 3, n = 63, BW = 404.4 ± 26.14 kg). Angus and Angus crossbred heifers were stratified by BW and allocated randomly to the following experimental treatments; 1) fed 1.8 kg/d barley grain concentrate (Barley); or, 2) fed 1.8 kg/d of high linoleic cracked safflower seed (Safflower). Heifers were fed 35 and 29 d, Exp. 1, Exp. 2 and 3. Following the feeding period, heifers (Exp. 1 and 2) were artificially inseminated (AI) 12 h after the onset of estrus, and heifers that did not show estrus were administered 25 mg of $PGF_{2\alpha}$ and time AI. A 7-d CO-synch + CIDR and TAI protocol was used for heifers in Exp. 3. Performance variables included initial and final BW, live wt gain, ADG, and DMI (Exp. 1 and 2). Biweekly blood samples were assayed for progesterone (P4) and $PGF_{2\alpha}$ metabolites (PGFM) concentrations (Exp. 1). Estrus responses were observed using Estrotect tags, heifers with progesterone (P4) < 1 ng/mL were not included in the calculation. Pregnancy rates were determined 40 d post breeding. Performance variables did not differ between barley and safflower treatments with the exception of DMI in Exp. 2. Heifers supplemented with barley consumed 21% more ($P < 0.001$) DM per d than heifer fed safflower. In Exp. 1, overall pregnancy rates were greater in safflower supplemented heifers than in barley supplemented heifers ($P = 0.036$). Pooled PGFM concentrations differed by day ($P < 0.05$) in Exp. 1. Results suggest supplemental high linoleic safflower seed does not appear to influence estrus response or PGFM concentrations in pubertal beef heifers.

CHAPTER 1

INTRODUCTION

The most important factor affecting profit in a cow-calf operation is reproduction (Hess. 2003). Increasing reproductive efficiency can be influenced by feeding dietary fat to increase energy density of the diet (Funston, 2004). However, there have been both positive and negative associated effects of fat supplementation on reproduction (Bottger et al., 2002; Funston, 2004; Scholljegerdes et al., 2009). Funston (2004) suggested that responses on reproductive tissues (hypothalamus, anterior pituitary, ovary and uterus) are dependent on the types of fatty acids present in the fat source. It is known that the use of polyunsaturated fatty acids in the diet of the cow influences luteal function either by direct action on progesterone production, or modification of eicosanoid (e.g. arachidonic acid) production (Mattos et al., 2000). Prostaglandin $F_{2\alpha}$ is known to have an inhibitory effect on progesterone production and can affect the length of the luteal phase (Lauderdale, 1974). Luteal regression in domestic ruminants is caused by uterine secretion of $PGF_{2\alpha}$. Infusion of linoleic acid into cattle gives rise to increased circulating concentrations of both $PGF_{2\alpha}$ and its metabolite 13, 14-dihydro-15-keto-PGF2α (PGFM; Juchem et al., 2010). Consequently, energy dense lipid supplements have evolved into a management strategy that may enhance reproductive function (Hess et al., 2005; Scholljegerdes et al., 2009).

Therefore, the focus of this project was to determine if the distribution of estrus can be altered by supplementing high linoleic acid safflower seed in diets of beef heifers,

and if circulating concentrations of PGFM are altered during and after dietary supplement with high linoleic safflower seed.

CHAPTER 2

LITERATURE REVIEW

Beef Cattle Production: Importance of Calf Production

In the past, when producers wanted to produce more beef, they would simply purchase more land and cows (Field, 2007). However, Field (2007) states the emphasis in today's cow–calf industry has shifted to increasing the productivity and profitability from the cow, acre, and total operation.

One way a cow-calf producer can increase profit and minimize loss is to focus on increasing pounds of production and income, while decreasing overall cost. To sustain a cow-calf operation, the majority of the income must come from the sale of the calf being produced. A breakeven price can be a simple way to look at profitability of a cow-calf operation (Field, 2007). The breakeven price analysis evaluates annual cow cost compared to pounds of calf weaned per cow (Field, 2007). The number of pounds of calf weaned per cow reflects both weaning weight and percent calf crop weaned per cow exposed (Field, 2007).

Change in weaning weight is one of the variables affecting income to a cow-calf producer. Use of an estrus synchronization protocol can be an effective method to increase the pounds of calf weaned per cow by shortening the breeding season and allowing for older and heavier calves at weaning time (Odde, 1990; Rodgers et al., 2012). A study conducted by Rodgers et al. (2012) concluded that cows exposed to estrus

synchronization plus artificial insemination programs produced $49.14 greater income than cows bred by natural service, with ending weaning weights as a main driving force.

Hess (2003) implied that the largest losses of income to cow-calf producers were attributed to female infertility and failure of cows to produce a viable calf. Failure of cows to become pregnant or calf loss after birth can account for 82% of the reduction in net calf crop (Field, 2007). Bellows et al. (2003) stated that the cost of female infertility in cows and heifers includes the cost of replacement of animals culled, in addition to any treatment and/or prevention expenses. Therefore, in order for cow-calf producers to minimize loss and sustain the operation, each cow needs to wean a calf each year.

Economic Importance of Reproduction in Beef Cattle

The goal of a cow-calf producer is to maintain the cowherd and for every cow to raise her calf from birth to weaning. Hess (2003) stated that reproductive efficiency of the cow herd is the most important factor in total calf production. Reproductive efficiency in cattle is measured by the number of calves born and weaned each year per hundred females in the herd, and is considered the most important economic factor in cattle production (Field, 2007). However, reproductive diseases and conditions can negatively affect calf production by delaying production and increasing treatment and management costs (Bellows et al., 2003). One of the greatest losses in calf production is due to decreased conception rates in beef cows at the end of the breeding season, which is most common in younger beef cows (Patterson et al., 1992; Bellows et al., 2003).

A two-year old cow is generally the most expensive and valuable animal of a ranch based on the amount of money invested into her and because she has not returned income to the operation (Geary, 2003). Geary (2003) estimated that it costs producers $950 to develop each replacement heifer and carry her through until calving. Replacement heifers must calve by 24 months of age to maximize reproductive efficiency within the herd (Patterson et al., 1992). Yet, many factors, such as nutrition and physiological state, can delay puberty and limit breeding (Short and Bellows, 1971; Patterson et al., 1992). Heifers fed more feed from 7 to 12 mo of age reached puberty sooner, and had increased pregnancy rates (Short and Bellows, 1971). Heifers that also calved early in the calving season have a longer post-calving interval before the second breeding (Dunn and Kaltenbach, 1980; Patterson et al., 1992). These young females require more intense management practices such as additional feed input and longer intervals before rebreeding to improve reproduction efficiency (Short and Bellows, 1971, Dunn and Kaltenbach, 1980; Patterson et al., 1992). Yet, they are essential to replenish the overall herd.

Factors that Affect Reproduction in Beef Cattle

Reproductive Diseases and Dysfunctions

Reproductive dysfunctions and diseases cost 3.4 to 3.9% of beef cow-calf value of production (Bellows et al., 2003). The majority of the cost from reproductive diseases and dysfunctions can be attributed to infertility and the failure for the cow to produce a healthy calf within the first 24 hours of life (Bellows et al., 2003). Reproductive diseases

and dysfunctions include viral and bacterial diseases that can cause abortions and dystocia, retained placentas, metritis and pyometra, and female infertility (Bellows et al., 2003).

Another common reproductive dysfunction is the inability of beef cows and heifers to become pregnant (Short et al., 1990). Geary (2003) stated that the cause of infertility in young beef cows is due to producers trying to rebreed a cow that has not yet reached her mature weight and cannot consume enough energy to satisfy growth, lactation, and maintenance demands. In order for heifers to calve early in their first calving season, they must reach puberty and become pregnant by about 15 mo. of age (Patterson et al., 1992). Thus adequate development of young heifers becomes vital for longevity. In growing heifers, the reproductive organs are the last to develop (Patterson et al., 1992). Excellent herd management and proper management of body condition, energy balance, and stress is essential for overall profitability in a beef cattle operation.

Nutrition

The importance of proper nutrition for livestock is recognized as the one of the most important factor to achieve optimal reproductive success (Wiltbank et al., 1962; Short et al., 1990; Dunn and Moss, 1992). Inadequate nutrition can lead to a reduction in energy reserves, thus redirecting available energy to overall maintenance of the animal, and restricting reproductive hormone secretion, such as GnRH (Short et al., 1990). Proper management practices and decisions, such as providing adequate nutrition, can minimize reproductive losses and optimize reproductive rates (Short et al., 1990). Jones et al. (2008) stated that a cow's body condition, energy balance, and stress can all be

influenced by nutrition. Sound nutritional programs are critical to achieve the highest reproductive rates and increase efficiency of beef cattle production (Hess, 2003).

A cow with a body condition score (BCS) less than 5 at calving can negatively affect a cow's reproductive function by delaying estrus after calving, and delaying pregnancy (Richards et al., 1986). Negative energy balance in cows can also result in decreased luteinizing hormone secretion and inhibit estrus resulting in a longer postpartum interval to estrus and a delayed re-breeding (Lucy et al., 1992). Lucy et al. (1992) reported that dairy cows before ovulation with a negative energy balance had slower growing preovulatory follicles compared to lactating dairy cows with a positive energy balance. Cows that are in good body condition at calving are the least affected by pre or postpartum weight changes (Dunn and Kaltenbach et al., 1980).

Cattle must consume adequate levels of feedstuffs in order to deliver the required nutrients for adequate production, such as body temperature regulation, essential metabolic processes, and physical activity, before energy is diverted to reproduction (Short et al., 1990). Reproduction is known to increase the net energy demand and can negatively affect a cow's energy balance. Dietary fat can be added to cattle diets to increase total energy. However, fats can also have a positive influence on reproduction in beef cattle by increasing precursors for the synthesis of reproductive hormones such as steroids and prostaglandins (Mattos et al., 2000; Wathes et al., 2007).

High quality forage and digestible cereal grains can increase total digestible energy (TDN) consumed in the diet which can increase the total energy available for gestation and lactation (NRC, 2000). However, adding feedstuff high in TDN can have

negative effects such as decreased digestion and utilization of nutrients (Bowman et al., 1996). Adding a fat supplement to a cow's diet can increase the energy density of the basal diet without having negative effects on digestion, milk composition, and health (Staples et al., 1997). Supplementing cows with fatty acids can increase body condition score, stimulate ovulation (Lucy et al., 1992), and increase the number of cows pregnant early in the breeding season (Alexander et al., 2002).

Fats, also known as lipids, contain more energy per pound than carbohydrates. Adding fat to a diet increases energy uptake and can affect energy balance in a positive way in cattle (Grummer et al., 1991). Sources of supplemental fats include yellow grease, fishmeal, cottonseed, soybean, canola seed, sunflower seed, safflower seed, and calcium soap of fat (Funston, 2004). Supplementing with soybean oil at 3% of forage-based diets increased ADG and feed efficiency in prepubertal beef heifers (Whitney et al., 2000).

However, supplementing cows with fat to increase the total energy intake can increase total feed costs and labor input. Beef producers should take the time to balance the diet to determine the nutrient requirements and whether a fat supplement is actually needed. Hess et al. (2008) summarized possible complications when supplementing cattle with fat to increase energy intake. Hess et al. (2008) indicated that an optimal inclusion rate for supplemental fat to be less than 3% of total DM in order to maximize the forage digestibility in the diet, and fat supplementation should not exceed 4% of DMI if the goal is to increase dietary digestible energy.

Lipids are either solid or an oil at room temperature, and can be either saturated or unsaturated depending on their particular chemical composition (Field, 2007). Lipids are

comprised of carbon, hydrogen, and oxygen molecules, and are soluble in organic solvents. Lipids include cholesterol, triacylglycerols, and phospholipids. Phospholipids are major components of cellular membranes and are a source of fatty acids for the synthesis of a variety of effector molecules such as eicosanoids.

Fats are glyceride esters of fatty acids and are important sources of energy. The function of a fatty acid is determined by the length of the acyl chain, number of double bonds in the chain, and the type of isomer formed by each double bond. Fatty acids are either synthesized in the body and/or are absorbed from the diet. Most fatty acids can undergo elongation and desaturation to generate fatty acids with different biochemical properties. Elongation involves adding two carbon units to the carbon chain. Desaturation is a process caused by desaturase enzymes that insert a double bond in the acyl chain. Fatty acids cannot undergo desaturation to form n-3 and n-6 families, so these are considered essential and must be provided by the diet. For example, linoleic acid is a known essential fatty acid that is required for the synthesis of arachidonic acid and eicosanoids (Mattos et al., 2000).

The essential fatty acids, n-3 and n-6 fatty acids, have biological functions in the ruminant such as being utilized for normal cellular functions, reversing a negative energy balance, and acting as a precursor to eicosanoids and steroid hormones (Jones et al., 2008).

The major fatty acid in most seed lipids is linoleic acid (Funston, 2004). Linoleic acid is a member of the n-6 family of fatty acids. Linoleic acid must be obtained through the diet because it cannot be synthesized de novo by any vertebrates (Lands, 1992).

Linoleic acid is available in numerous sources such as soybean, safflower, and sunflower seed. Linoleic acid has 18 carbon atoms and two double bonds, with its first double bond at the sixth position from the methyl end. Linoleic acid can be desaturated and elongated to form arachidonic acid, which is a precursor for prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). The conversion of linoleic acid to arachidonic acid involves two steps of desaturation at the 5 and 6 positions, and 2 carbon elongations. Arachidonic acid is stored within phospholipids of cellular plasma membranes from which it can be released and processed by enzymes to produce prostaglandins.

Linoleic acid is a precursor to group 2 eicosanoids, and group 2 eicosanoids can benefit the overall health in a cow (Jone et al., 2008). Holstein cows supplemented with linoleic and linolenic acids had fewer instances of metritis and other uterine abnormalities (Jones et al., 2008). Hess (2003) stated that feeding a fat supplement for 60 days before parturition resulted in a 6.4% pregnancy rate improvement. Supplemented fats can even play an important role in reproduction by aiding in the stimulation or inhibition of reproductive hormones (Jones et al., 2008) such as $PGF_{2\alpha}$ synthesis.

Nutritional Effects on Reproductive Hormones

There are mixed outcomes documented regarding the effects supplemental fats have on reproductive hormones. Funston's (2004) review on fat supplement and reproduction elucidated that the reason for the varied and inconsistent results could be due to the complexity of the reproductive system and the makeup of fat supplement, which could be confounded by management practices and forage quality. Mattos et al.

(2000) stated that an increased availability of fatty acid precursors would increase steroid and eicosanoid secretion. An increase in eicosanoid secretion in the blood and could alter ovarian and uterine function and hence affect pregnancy rates in beef cattle.

Short and Adams (1988) suggested that there is a relationship between the neural-hormonal control of reproduction and energy intake specifically glucose. Glucose is the only energy source used by the central nervous system and therefore is the specific mediator for the effects of energy intake on reproduction (Short and Adams. 1988). The secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus plays an important role in reproduction and the initiation of estrus. However, Funston (2004) stated that measuring metabolites may not be accurate due to the difference in clearance rate or cellular uptake that could occur with lipid supplementation.

The secretion of LH from the pituitary and follicular growth in cattle is partially regulated by the energy status of the animal (Mattos et al., 2000). Fat supplementation can increase LH secretion when the animal is deficient in energy, but whether fatty acids affect LH secretion has not been established (Mattos et al. 2000). Follicular dynamics, such as follicular waves and development of ovarian follicles, are linked to a cow's energy balance, but can be influenced with dietary fat (Lucy et al., 1992, Jaiswal et al., 2009). An increase in growth, total number, and size of preovulatory follicles have been reported for cows supplemented with long chain fatty acids (Lucy et al., 1992; Mattos et al., 2000). Ovulation of a larger dominant follicle may result in the formation of a larger corpora lutea with possibility of greater progesterone production (Funston, 2004).

Dietary fat supplementation has been shown to increase circulating concentrations of progesterone and the lifespan of corpus luteum in cattle (Staples et al., 1998; Funston, 2004). Yet, there are conflicting results linking n-3 and n-6 polyunsaturated fatty acids direct effects on progesterone secretion (Staples et al., 1998; Mattos et al., 2000; Robinson et al., 2002; Gulliver et al., 2012). Generally, a diet high in n-6 polyunsaturated fatty acid increases cholesterol concentrations, and since cholesterol acts as a precursor to progesterone, progesterone concentrations usually increase (Staples et al., 1998; Gulliver et al., 2012). Another reason for increased circulating progesterone concentrations is the effect n-6 fatty acids may have on $PGF_{2\alpha}$ by depressing production and allowing for a greater progesterone synthesis and secretion (Staples et al., 1998).

Robinson et al. (2002) reported a reduction in systemic progesterone concentrations in lactating dairy cows supplemented with n-3 or n-6 polyunsaturated fatty acids. The four possible explanations for the decreased progesterone concentrations in the lactating dairy cows supplemented with polyunsaturated fatty acids are: 1) polyunsaturated fatty acids can alter prostaglandin synthesis; 2) an influence of arachidonic acid on progesterone production; 3) increased cholesterol concentrations; and 4) a delayed ovulation resulting in delayed luteal development (Robinson et al., 2002). This reduction of progesterone levels in the bloodstream, due to fat supplementation, can have a negative effect on the fertility and reduce pregnancy rates (Robinson et al., 2002).

Prostaglandin synthesis relies on the release of its precursor fatty acid, arachidonic acid, to be processed by enzymes. Arachidonic acid can either by synthesized from linoleic acid or acquired from the diet. Cheng et al. (2001) stated that feeding diets

high in n-6 polyunsaturated fatty acids can either inhibit or stimulate prostaglandin synthesis. Numerous studies (Gummer et al., 199; Alexander et al., 2002; Grant et al., 2003; Hess et al., 2005) have reported an increase in prostaglandin synthesis in cows supplemented with high-linoleic fat. Hess et al. (2005) reported an increase in $PGF_{2\alpha}$ metabolite when cows were supplemented with fat. This increase could be due to linoleic acid, once desaturated and elongated to arachidonic acid, serving as a precursor to $PGF_{2\alpha}$ production (Funston, 2004). Other researchers (Talavera et al., 1985; Staples et al., 1998; Matto et al., 2000; Cheng et al., 2001) have reported a decrease in prostaglandin synthesis when cattle were supplemented with polyunsaturated fatty acids. In a review article by Mattos et al. (2000) five potential methods by which supplemental fatty acids can inhibit $PGF_{2\alpha}$ secretion were discussed. These five possible methods were: 1) decreased synthesis of arachidonic acid; 2) competition of the n-3 fatty acids for desaturase activity; 3) alteration of the fatty acid profile in the plasma membrane; 4) competition of polyunsaturated fatty acids with arachidonic acid for prostaglandin H synthetase; and 5) inhibiting prostaglandin H synthetase and activity. Excess polyunsaturated fatty acids in the diet can inhibit the synthesis of arachidonic acid (Staples et al., 1998) and prevent arachidonic acid from desaturation and elongation into 1 and 2 prostaglandins (Matto et al., 2000). The presence of high n-3 linolenic fatty acid can also compete with n-6 linoleic acid for binding with delta 6-desaturase and reduce arachidonic acid production (Staples et al., 1998; Matto et al., 2000). The increased absorption of n-3 fatty acids from a diet can also reduce availability of arachidonic acid, yet increase other C-20 fatty acid availability such as eicosapentaenoic acid (Matto et al., 2000). With less arachidonic acid

to compete with the other 20-carbon fatty acids for active sites on the prostaglandin-endoperoxide synthase complex, the conversion of arachidonic acid to $PGF_{2\alpha}$ is lessened (Staples et al., 1998). Instead of competing, eicosapentaenoic acids and other C-20 fatty acids are thought to inhibit conversion of arachidonic acid to $PGF_{2\alpha}$ (Matto et al., 2000). However, inhibiting the production of $PGF_{2\alpha}$ may be a useful tool for manipulating reproductive processes in beef cattle.

The Estrous Cycle in Beef Cattle

The average length of the estrous cycle in cattle is 21 days. Estrus begins with an increase in estrogen concentrations in the blood which causes the cow to display behavioral signs of heat and also stimulates a surge of gonadotropin releasing hormone (GnRH). The surge of GnRH stimulates the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) that results in dominant follicle rupture and release of an oocyte into the oviduct approximately 28 hours after the onset of estrus. In the five days following ovulation, the cells in the follicular cavity will luteinize to form the corpus luteum (CL). The CL secretes progesterone (P_4) to prepare the uterus for pregnancy and inhibits the release of GnRH, and LH. If an embryo is not present by day 17 of the estrous cycle, the uterus will begin to secrete prostaglandin $F_{2\alpha}$ to stimulate the regression of the CL, and a decrease in circulating P_4 concentrations in the bloodstream which allows a dominant follicle to mature, produce estradiol and initiate the next estrous cycle.

Estrous Synchronization in Beef Heifers

Synchronization of estrous cycles has the potential to achieve high pregnancy rates with a shortened breeding season, increasing calf uniformity, as well as facilitating the use of AI. Estrus synchronization involves the use of prostaglandins to manipulate the CL and the estrous cycle of females within a herd so they can express estrus at approximately the same time (Geary, 1997). However, estrous synchronization can also increase production costs and labor input.

The success of a synchronization program depends on many factors including facilities, available labor, the body condition, post calving interval, fertility level of the cows and heifers (Field, 2007), and understanding how it works (Geary, 1997). However, one of the most serious considerations of an estrous synchronization program is selecting a proper estrous synchronization method or protocol that increases the chances of all cows to respond and express estrus (Geary, 1997; Field, 2007).

A proper estrous synchronization protocol can effectively shorten the breeding season to 45 days (Odde, 1990); thus resulting in calves being born earlier and resulting in an older, heavier, and more uniform calf crop (Odde, 1990). Estrous synchronization protocols that result in a highly synchronized estrus and ovulation reduce the time and labor associated with estrus detection, thereby making estrus synchronization more feasible to a broader range of producers (Mallory et al., 2010).

As indicated earlier in this review, 2 yr old cows and heifers are the most expensive to maintain, but are valuable because the producer has yet to make a return from the heifer (Geary, 2003). An estrous synchronization program for replacement

heifers can offer many benefits to beef producers, such as increased rebreeding pregnancy rates (Geary, 2003).

Numerous researchers (Odde, 1990; Patterson et al., 1992; Geary, 2003; Lamb et al., 2006; Hall et al., 2009; Mallory et al., 2010) agree that an estrous synchronization program can enhance reproductive performance by increasing the number of heifers calving early in the calving season, tightening calving intervals, increasing rebreeding pregnancy rates, facilitating use of AI, and even reducing dystocia. Using both an estrous synchronization protocol and AI can assist beef producers to avoid dystocia by using calving ease proven sires for breeding heifers (Geary, 2003).

However, some disadvantages of estrous synchronization protocols include the cost of pharmaceuticals, increased time and labor requirements, and failure to synchronize all females in the herd. The current commercial price quote from veterinary supply companies for a controlled internal drug releasing (CIDR) is $10.80 per insert, prostaglandins, $2.84 to $3.09 per dose, and GnRH, $2.66 to $3.06 per dose. Some estrus synchronization protocols require multiple doses of these drugs. In order to administer these protocols, cows have to be handled and processed multiple times, which can increase the time and labor costs

Estrous Synchronization Protocols

All estrous synchronization protocols use a $PGF_{2\alpha}$ injection to control CL lifespan. However, the CL is only responsive to a single injection of $PGF_{2\alpha}$ from d 5 to d 17 of the estrous cycle. Thus, heifers that are prepubertal or are not between d 5 and d 17

of their estrous cycle will not respond to an injection of $PGF_{2\alpha}$. In order to manipulate estrous cycles of heifers and induce an estrous cycle in prepubertal heifers, other reproductive hormone products, such as progestins and/or GnRH, must be utilized. Progestins include the progesterone-like feed additive melengestrol acetate (MGA) and CIDR device, which is impregnated with progesterone and can be inserted into the vagina of heifers. Progestins are known to suppress LH release (Anderson et al., 1996) and are used to mimic a luteal phase until removed from either the feed or from the female. While GnRH is capable of inducing estrous cycles in heifers, it is used more often to manipulate follicular turnover (Thatcher et al., 1989; Pursley et al., 1997). The tightest synchronization of estrus in heifers is achieved by manipulating both CL lifespan and follicular development. Several of the more common protocols for synchronizing estrus in heifers are described below.

In this protocol, all heifers receive one injection of $PGF_{2\alpha}$, and are observed for estrus for 4 to 5 d after the injection, and are bred 12 hours after standing estrus (Lauderdale et al., 1974). Greater than 75 % of the pubertal heifers should be expected to exhibit estrus by d 7 after the injection (Lauderdale et al., 1974). However, this protocol only works in cycling females, and does not induce puberty in heifers.

In this protocol, oral progesterone analog, melengestrol acetate (MGA), and a prostaglandin injection are used. This is a low cost easily administered synchronization technique (Lauderdale, 1975). MGA is fed at 0.5mg/head/day for 14 days, and a single injection of $PGF_{2\alpha}$ is administered 17 to 19 d after the MGA removal (Patterson et al., 1992; Lamb et al., 2001). Most heifers will exhibit estrus 48 to 72 h after the $PGF_{2\alpha}$

injection and should be bred 12 h after observed estrus (Patterson et al., 1992; Geary, 1997). The advantages to this synchronization protocol is the decrease in cost and labor, fewer days of estrous detection and trips through the chute, and can induce puberty in prepubertal heifers (Patterson et al., 1992). However, MGA requires daily feeding and all animals must consume the recommended amount per day, and this protocol takes at least 31 days to synchronize the herd (Patterson et al., 1995).

The Controlled Internal Drug Release (CIDR) is an insert device used for dispensing progesterone and can be used in conjunction with other protocols that use GnRH and $PGF_{2\alpha}$ to synchronize estrus in female cattle (Peel et al., 2013). The CIDR device is a rubber T-shaped device that is placed in the vagina for 5 to 14 days. This device works well on anestrous females and ensures every female receives the same progestin exposure, but the cost may be hard to justify for cycling females (Johnson et al., 2005).

Generally a $PGF_{2\alpha}$ injection can be administered either a day before or the day of the CIDR removal (Lucy et al., 2001). Estrus should be detectable within 1 to 3 days after CIDR removal (Lucy et al., 2001). These two CIDR protocols can induce puberty in heifers and estrus in non-cycling cows, and decrease the days of estrus detection (Field, 2007; Hall et al., 2009). This protocol has a high drug cost and requires multiple trips through a chute (Hall et al., 2009). Recent research has concluded no improvement in estrus synchronization or AI pregnancy rates in protocols that require an injection of GnRH before the $PGF_{2\alpha}$ injection (Peel et al., 2013).

CO-Synch + CIDR protocol combines the injections of the GnRH and $PGF_{2\alpha}$, with either a 5 d or 7 d CIDR insert (Peel et al., 2013). All heifers receive a GnRH injection and a CIDR insert on d 0, the CIDR is removed and PG is administered to all females on d 5 or d 7. A second injection of GnRH is administered 48 to 72 h later in conjunction with mass breeding of heifers that did not display estrus (Peel et al., 2010). With the 5 d CO-Synch + CIDR protocol, some studies have revealed improved results with a second injection of $PGF_{2\alpha}$, 8 to 10 h after the removal of the CIDR (Bridges et al., 2012).

A long term protocol that combines a 14 d CIDR plus an injection of $PGF_{2\alpha}$, followed by another injection of GnRH 66 to 68 h later has proven to be an effective synchronization protocol for heifers (Tauck et al., 2007).

Utilizing Fatty Acids for Improvement of Reproduction

Numerous studies have resulted in positive outcomes for cows fed supplemental fat on energy balances (Grummer et al., 1991). Feeding supplemental fat increases the energy intake which can reverse a negative energy balance and improve reproductive efficiency. Yet, some research, suggested that this is probably not a result of improvement of the energy status of the cow, but due to effects of dietary fatty acids on the pituitary, ovaries and uterus (Mattos et al., 2000). Cows supplemented with fatty acids had fewer instances of metritis and other uterine abnormalities, resumed cyclicity sooner after parturition, and exhibited improved uterine health and fertility (Jones et al., 2008). One study determined that feeding ovariectomized ewes flax seed resulted in

lower clearance rates of circulating progesterone compared to ewes fed diets lower in fatty acids which improve pregnancy rates (Galbreath et al., 2008). Galbreath et al. (2008) suggested that lower progesterone concentrations in ewes could be due to the shift from progesterone metabolism to prostaglandin synthesis when fatty acids are increased in the diet. Yet, Talavera et al. (1985) observed an increase in luteal progesterone biosynthesis in dairy heifers fed a fatty acid supplement. Some reviewed articles related to the effects of dietary fat on reproduction, have reported improvement in first service conception and pregnancy rates in cattle fed supplemental fats (Staples et al., 1998; Alexander et al., 2002). Staples et al. (1998) associated improved embryo survival rates in dairy cows with the reduction of $PGF_{2\alpha}$ secretion due to supplementing fatty acids. Lucy et al. (1992) determined that feeding additional long chain fatty acids stimulated the size and development of preovulatory follicles. The ovulation of larger follicles may result in the formation of a larger corpora lutea with increased steriodogenic capacity (Lucy et al., 1992; Mattos et al., 2000). All these outcomes indicate that fatty acids have the potential to improve uterine health, follicular development, and pregnancy rates. These results could be beneficial to livestock producers to increasing reproductive efficiency of the total herd.

However, supplementing with fat has produced some negative results such as decreased first conception rate and overall pregnancy rate (Lucy et al., 1992). Grant et al. (2002) reported an increased $PGF_{2\alpha}$ synthesis when supplementing high linoleic fat to cows for extended period of time, resulted in shorter estrus cycles and compromised embryo survival (Grant et al., 2003).

Alexander et al. (2002) hypothesized that the beneficial effect of fat supplementation on reproduction performance would be masked when cattle are supplied with more than adequate amounts of nutrients by the basal diet and other dietary factors may need to be considered. Whereas, Funston (2004) concluded from other studies that there are limited benefits to improve reproduction efficiency by supplementing fat to well-developed replacement heifers.

Utilizing Linoleic Acid for Estrous Synchronization

There are limited synchronization protocols that do not require beef producers to gather the herd for hormone injections. MGA is the only synchronization drug that can be administered orally. However, MGA is regulated by FDA, and is most effective when combined with a PG injection after the feeding period. Limited research has been conducted with utilizing fat supplementation to synchronize estrus, but much research has been conducted on the effects of high linoleic acid on reproduction performance in beef cattle (Staples et al., 1998; Cheng et al., 2000; Bottger et al., 2002; Hess, 2002; Funston, 2004; Scholljegerdes et al., 2009). According to a review by Funston (2004), fat supplementation has positively affected reproductive functions on several important tissues, including the hypothalamus, anterior pituitary, ovary, and uterus. These target tissues and reproductive responses seem to be dependent on certain fatty acids (Funston, 2004). Wathes et al. (2007) suggests that n-3 and n-6 polyunsaturated fatty acids can directly or indirectly exerted different effects on ovarian steroid synthesis. One study conducted with Holstein heifers resulted in elevated P_4 concentrations when the heifers

were fed a sunflower supplement diet (Talavera et al., 1985). Furthermore, results of Cheng et al. (2001) indicated that a diet high in n-6 polyunsaturated fatty acids can reduce endometrial capacity to produce prostaglandin and may have implications for the control of luteolysis and ovulation. In contrast, Hess et al. (2005) reported greater concentrations of $PGF_{2\alpha}$ in cows fed supplemental fat high in linoleic acid. Whether supplementing high linoleic acid inhibits or enhances $PGF_{2\alpha}$ concentrations, development of an estrous synchronization protocol based on high levels of linoleic acid in feedstuff could obviate the need for labor-intensive estrus synchronization protocols.

CHAPTER 3

EFFECTS OF FEEDING HIGH LINOLEIC ACID SUPPLEMENT ON THE DISTRIBUTION OF ESTRUS AND TEMPORAL CONCENTRATIONS OF PGFM IN BEEF HEIFERS

Introduction

An important factor affecting profit in a cow-calf operation is the ability for each cow to produce a viable calf (Hess, 2003). This goal is even more difficult to achieve in younger beef cattle, especially heifers, due to increasing energy demands for growth and maintenance. Increasing reproductive efficiency can be influenced by feeding dietary fat to increase energy density in the diet. Not only does increasing dietary fat reverse negative energy balances, but previous studies (Bottger et al., 2002; Scholljegerdes et al., 2009) have associated both positive and negative effects of fat supplementation on reproductive functions. Reproductive tissues such as the hypothalamus, anterior pituitary, ovary and uterus may be more sensitive to n-6 fatty acids present in the fat source than has currently been indicated. It is known that fatty acids are essential precursors for the synthesis of a variety of molecules like eicosanoids, which include prostaglandins. Results of studies have shown that supplementing fat in the form of high linoleic acid can result in increased concentrations of $PGF_{2\alpha}$ (Hess et al., 2005) which can effect P_4 production and, perhaps, manipulate estrous response (Mattos, et al., 2000; Funston, 2004; Juchem et al., 2010). The concept of using supplemental fat as a management strategy to enhance reproductive efficiency and possibly manipulating the length of the estrous cycle continues to stimulate interest, especially in beef heifers. Beef

producers need to breed their heifers by 15 months of age to calve by 2 years of age and heifers that calve early in the calving season have greater lifetime productivity (Patterson et al., 1992). Thus, if a simple management protocol consisting of supplementing fat to beef heifers could be developed that improves synchrony of estrus and fertility, could be beneficial to increasing reproductive efficiency.

The null hypotheses that were tested in this study were: 1) feeding a dietary supplement high in linoleic acid to cycling beef heifers would not influence the distribution of estrus after the removal of the supplement; 2) feeding a dietary supplement high in linoleic acid to cycling beef heifers would not influence systemic concentrations of PGFM during and after the removal of the supplement. The first objective of this study was to determine if estrous synchronization in beef heifers could be achieved by the addition of high levels of linoleic acid in the diet. The second objective was to determine if high levels of linoleic acid in a dietary supplement would alter concentrations of PGFM during and after dietary supplement removal.

Materials and Methods

Exp. 1

Study Animals. All procedures were approved by the Montana State University Agriculture Animal Care and Use Committee (AACUC). The experiment was conducted at the Montana State University Bozeman Agricultural Research and Teaching Farm (BART). Forty-eight spring-calving (12 mo of age) Angus x Hereford beef heifers considered to have reached puberty, that had a functional CL, and progesterone

concentrations greater than 1 ng/mL were stratified by BW (370 ± 24.8 kg) and randomly assigned to 1 of 2 dietary treatments.

Dietary Treatments. Treatments consisted of chopped grass hay (CP = 8%; NDF = 65%; ADF = 45%, DM Basis; chopped to pass a 10 cm screen) mixed with one of two supplements: 1) 1.8 kg/d per head of barley grain concentrate (Barley; n = 24); and 2) 1.8 kg/d per head of high linoleic cracked safflower seed (Safflower; n = 24). Prior to study, heifers were allocated to one of four pens with twelve animals per pen. Heifers were allowed ad libitum access to fresh water. Diets were fed twice daily at 0800 and 1600 for 35 d (Figure 1). Pre-experimental analysis determined that the safflower seeds contained 17.1 % CP and 53% TDN and the barley grain contained 15.0% CP and 81% TDN on a DM basis (Table 1). Safflower seeds were cracked with a hammer mill before mixing into the diet. The dietary amount of the safflower seeds were provided at levels similar to previously reported by Scholljegerdes et al. (2004). Diets were not formulated to be isonitrogenous or isoenergetic, but did meet the nutritional requirements of a 12-mo, 360 kg of BW pubertal heifer (NRC, 2000). Grab samples of each treatment diet were collected weekly for dry matter analysis.

Table 1. Ingredient composition and nutrient analysis (% DM) of barley and safflower treatments fed twice daily to beef heifers at BART[1] farm in Exp. 1

	Treatment	
Item	Barley	Safflower
Ingredient composition, kg/d		
Chopped grass hay	9.1	9.1
Barley mix	1.8	
Cracked safflower seeds		1.8
Chemical composition[2], % DM		
CP	15.0	17.1
ADF	5.3	31.5
NDF	16.5	46.0
TDN	81.0	53.0

[1]BART = Bozeman Agricultural Research and Teaching Farm.
[2]Chemical composition for barley mix and safflower seeds.

Data Collection. Heifers were weighed individually at allocation and d 0, 2, 12, 23, 35, and 41 thereafter (Figure 1). Each heifer was fitted with passive radio frequency transponder tag to measure individual intake, bunk attendance, and feed disappearance with the GrowSafe automated feed intake measurement system (GrowSafe Systems Ltd., Airdrie, Alberta, Canada) for the 35-d feeding period. Daily feed intakes were computed by using the Process Intakes routine of the GrowSafe Acquisition software. Feeding behavior traits were also computed using the Export Behavior data routine of the GrowSafe Acquisition software. Grab samples of diets were dried in forced- air oven for 24 h at 100° C using the AOAC (2000) procedure to determine the DM of each treatment diet.

Estrus and Pregnancy Detection. Each heifer was fitted with an Estrotect patch (Rockway Inc., Spring Valley, WI) to detect estrus during the feeding period and for 10 d after the removal of the treatment diets. The Estrotect patch is applied over the sacrum of

the animal (located on the line between the hipbones and the tail head). The detector remains white until it is triggered. Triggering takes place when the pressure from the brisket of a mounting animal turns the detector a bright pink. Estrotect patch color was recorded and replaced if the tag had changed color on d 0, 12, and 23 (Figure 1). All Estrotect patches were replaced at d 35 of the trial and heifers were monitored twice daily for estrus behavior from d 37 to d 41 of (Figure 1). Heifers were bred during this interval by a trained AI technician 12 h after the onset of estrus until d 41 (Figure 1). Heifers that did not exhibit estrus by d 41 were injected intramuscular (i.m.) with 25 mg of $PGF_{2\alpha}$ and bred by AI 12 h after estrus detection (d 41 to d 45; Figure 1). The uterus of each heifer was examined by rectal palpation for pregnancy 40 d post-breeding by licensed veterinarian.

Blood Sampling Procedures. Blood samples (~10 mL) were collected from one jugular vein of each heifer via venipuncture using 10-mL untreated Kendall Monoject Blood Collection (Tyco HealthCare Group LP, Mansfield, MA) tubes on d 0, 12, 23, 35 and 41(Figure 1). Samples were allowed to clot at 4°C for 24 h then centrifuged at 1,850 x g for 30 min. Sera was harvested and stored at -20°C until assayed for P4 and PGFM.

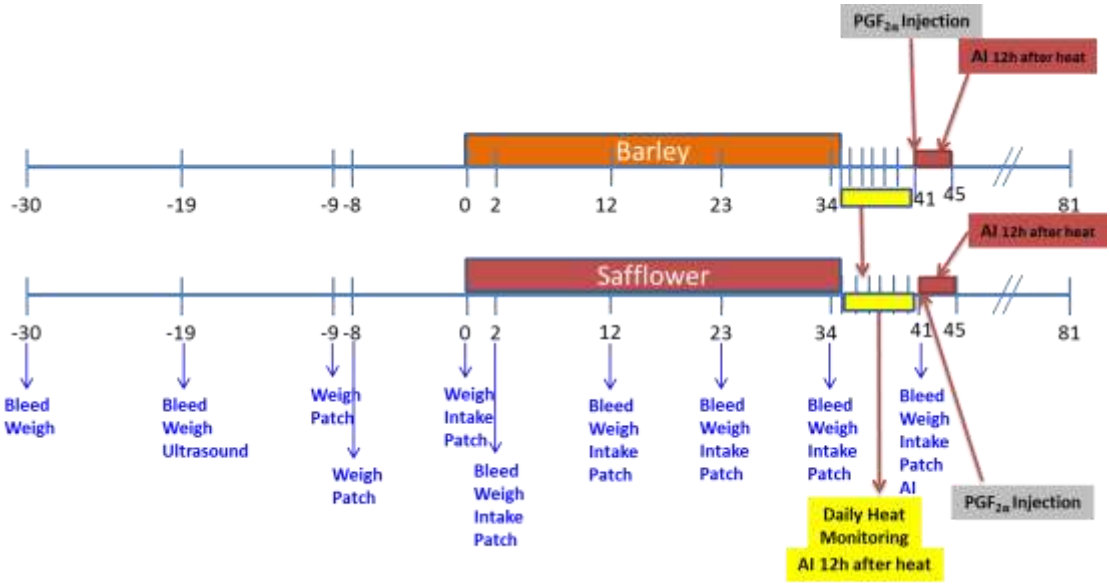

Figure 1. Timeline of samples and observations collected for both barley and safflower supplemented heifers at the BART farm (Exp.1).

Progesterone Assay. Progesterone concentrations were determined in serum samples in duplicate by a solid-phase RIA kit (Siemens Healthcare Diagnostics, Los Angeles, CA). The intra- and inter assay CV were 4.5 and 6.9%, respectively.

PGFM Assay. Heifer serum samples were sorted by treatment (n=2) and randomly assigned 6 heifers per tube (n=4) for d 2, 12, 23, 34, and d 41. Concentrations of PGFM in pooled serum samples were determined in triplicate using an ELISA kits (Arbor Assays, Ann Arbor, MI), with a minimum detection limit of 45.4 pg/mL. The intra- and inter-assay CV% were less than 14% in pooled heifer serum samples that contained 121 and 533 pg/mL.

Statistical Analyses. Data for performance and intake over the 34-d feeding period were statistically analyzed by ANOVA for a completely randomized design using the

GLM procedure of SAS (SAS Inst., Inc., Cary, NC). Least square means and associated standard errors were reported. Significance was set at an alpha level of 0.05.

Heifers were removed from summarized data if they were considered non-cyclic based on multiple progesterone concentrations of < 1 ng/mL collected during the dietary treatment period (d 0 to d 41). Estrous responses and pregnancy rates for barley- and safflower seed-supplemented heifers that were bred by AI 12 h after estrus (d 1 to d 6) after supplementation were analyzed separately by chi-square analysis using the PROC FREQ procedure of SAS. Artificial insemination (AI) pregnancy rates after PGF_{2a} for barley- and safflower seed-supplemented heifers that were not bred by AI (d 0 to d 6) after supplementation were analyzed by chi-square analysis using the PROC FREQ procedure of SAS (SAS Inst. Inc.). *P*-values were reported using Fisher's Exact Test, and significance was set at an alpha level of 0.05.

Pooled serum concentrations of $PGF_{2\alpha}$ metabolites sorted by treatment with 6 serum samples per tube (n =4) for d 2, 12, 23, 34, and 41 were analyzed by ANOVA using the PROC MIXED model for repeated measures of SAS. The model included treatment, day, and the treatment by day interaction. Heifer within treatment was the subject and day was the repeated measure. Means were separated using Bonferroni's multiple comparison tests and associated standard errors are reported. Significance was set at an alpha level of 0.05.

Exp. 2

Study Animals. All procedures were approved by the Montana State University Agriculture Animal Care and Use Committee (AACUC). The experiment was conducted at the Northern Agricultural Research Center (NARC) of Montana Agricultural Experiment station. Forty spring-born (12 mo of age) Angus x Charolais beef heifers that were considered to have reached puberty, based on a functional CL and progesterone concentrations greater than 1 ng/mL, were stratified by BW (396.5 ± 31.7 kg) and randomly assigned to 1 of 2 dietary treatments.

Dietary Treatments. Treatments consisted of 2.67 kg/d per head chopped alfalfa brome hay, 0.90 kg/d per head of chopped clover/alfalfa hay, 6.8 kg/d per head corn silage, 0.90 kg/d per head of barley straw, and 0.27 kg/d per head medicated protein supplement on as-fed basis and one of two supplement; 1) 1.8 kg/d per head of barley grain concentrate (Barley; n = 20); and 2) 1.8 kg/d per head of high linoleic cracked safflower seed (Safflower; n = 20). Prior to start of the feeding period, heifers were allocated to one of two pens with twenty heifers per pen. Heifers were allowed ad libitum access to fresh water. Diets were fed twice daily at 0800 and 1600 for 29 d (Figure 2). Pre-experimental analysis determined that the safflower seeds contained 17.1 % CP and 53% TDN and the barley grain contained 15.0% CP and 81% TDN on a DM basis (Table 2). Safflower seeds were cracked with a hammer mill before mixing into the diet. The dietary amount of the safflower seeds were provided at levels similar to previously reported by Scholljegerdes et al. (2004). Diets were not formulated to be isonitrogenous

or isoenergetic, but did meet the nutritional requirements of a 12 mo, 396 kg of BW pubertal heifer (NRC, 2000). Grab samples of each treatment diet were collected weekly for dry matter analysis.

Table 2. Ingredient composition and nutrient analysis (% DM) of barley and safflower treatments fed twice daily to beef heifers at NARC[1] farm in Exp. 2 and 3 [2]

	Treatments	
Item	Barley	Safflower
Ingredient composition, kg/d		
Chopped alfalfa brome hay	2.7	2.7
Chopped clover alfalfa hay	0.9	0.9
Corn Silage	6.8	6.8
Barley straw	0.9	0.9
NutraLix supplement	0.3	0.3
Barley mix	1.8	
Cracked safflower seeds		1.8
Chemical composition[3], % DM		
CP, % of DM	15.0	17.1
ADF, % of DM	5.3	31.5
NDF, % of DM	16.5	46.0
TDN, % of DM	81.0	53.0

[1]NARC = Northern Agricultural Research Center.
[2]Diet used in Exp. 2 and Exp. 3 conducted at the NARC farm.
[3]Chemical composition for barley mix and safflower seeds.

Data Collection. Heifers were weighed individually at allocation and on d 0,1, 8, 19, and 29 (Figure 2). Each heifer was fitted with passive radio frequency transponder tag to measure individual intake, bunk attendance, and feed disappearance with the GrowSafe automated feed intake measurement system (GrowSafe Systems Ltd., Airdrie, Alberta, Canada) for the 29-d feeding period (Figure 2). Individual data were recorded using GrowSafe Acquisition software. Daily feed intakes were computed by using the Process Intakes routine of the GrowSafe Acquisition software. Feeding behavior traits

were also computed by using the Export Behavior data routine of the GrowSafe Acquisition software. Grab samples of diets were dried in forced- air oven for 24 h at 100° C using the AOAC (2000) procedure to determine the DM of each treatment diet.

Estrus and Pregnancy Detection. Each heifer was fitted with an Estrotect patch (Rockway Inc. Spring Valley, WI) to detect estrus during the feeding period and each day for 4 of the 10-d breeding period. The Estrotect patch is applied over the sacrum of the animal (located on the line between the hipbones and the tail head). The detector will remain white until it is triggered. Triggering takes place when the pressure from the brisket of a mounting animal turns the detector a bright pink. Estrotect patch color was recorded and replaced, if the tag had changed color, d 0, 8, 19, and 29 (Figure 2). All Estrotect patches were replaced at d 29 of the trial and heifers were monitor twice daily for estrous from d 34 to d 37 (Figure 2). Heifers were bred during this interval by a trained AI technician 12 h after the onset of estrous (d 34 to d 37) (Figure 2). Heifers that did not exhibit estrus and AI by d 37 were injected i.m. with 25 mg of $PGF_{2\alpha}$ and bred by TAI 60 h later (Figure 2). The uterus of each heifer was examined by rectal palpation for pregnancy 40 d post breeding by licensed veterinarian.

Blood Sampling Procedures. Blood samples (~10 mL) were collected from one jugular vein form each heifer via venipuncture using 10-mL untreated Kendall Monoject Blood Collection (Tyco HealthCare Group LP, Mansfield, MA) tubes on d 0, 8, 19, and 29 (Figure 2). All blood samples were allowed to clot at 4°C for 24 h then centrifuged at 1,850 x g for 30 min. Sera was harvested and stored at -20°C until assayed for P_4.

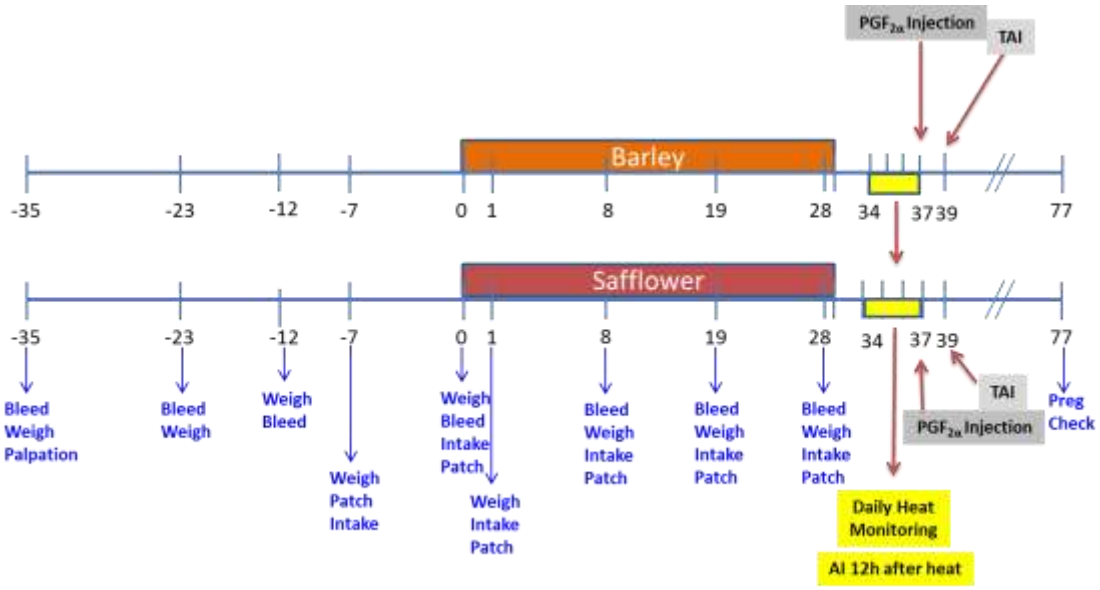

Figure 2. Timeline of samples and observations collected for both barley and safflower supplemented heifers at the NARC farm (Exp. 2).

Progesterone Assay. Progesterone concentrations were determined in serum samples in duplicate by a solid-phase RIA kit (Siemens Healthcare Diagnostics, Los Angeles, CA). The intra- and inter assay CV were 4.5 and 6.9%, respectively.

Statistical Analyses. Data for performance and intake over the 29-d feeding period were analyzed by ANOVA for a completely randomized design using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). Least square means and associated standard errors were reported. Significance was set at an alpha level of 0.05.

Heifers were removed from summarized data if they were considered non-cyclic based on multiple progesterone concentrations < 1 ng/mL collected during the dietary treatment period (d 0 to d 29). Estrous responses and pregnancy rates for barley- and safflower seed-supplemented heifers that were bred by AI 12 h after estrus (d 0 to d 4) after supplementation were analyzed separately by chi-square analysis using the PROC

FREQ procedure of SAS. Timed artificial insemination (TAI) pregnancy rates after PGF_{2a} for barley- and safflower seed-supplemented heifers that were not bred by AI (d 0 to d 4) after supplementation were analyzed by chi-square analysis using the PROC FREQ procedure of SAS (SAS Inst. Inc.). *P*-values were reported using Fisher's Exact Test, and significance was set at an alpha level of 0.05.

Exp. 3

Study Animals. All procedures were approved by the Montana State University Agriculture Animal Care and Use Committee (AACUC). The experiment was conducted at the Northern Agricultural Research Center (NARC) of Montana Agricultural Experiment station. Sixty-three spring-born (12 mo of age) Angus beef heifers considered to have reached puberty, that had a functional CL, and progesterone concentrations greater than 1 ng/mL were stratified by BW (404.4 ± 26.14 kg) and randomly assigned to 1 of 2 dietary treatments.

Dietary Treatments. Both treatments consisted of 2.67 kg/d per head chopped alfalfa brome hay, 0.90 kg/d per head of chopped clover alfalfa hay, 6.8 kg/d per head corn silage, 0.90 kg/d per head of barley straw, and 0.27 kg/d per head medicated protein supplement on as-fed basis and one of two supplements; 1) 1.8 kg/d per head of barley grain (control) concentrate (n = 31); and 2) 1.8 kg/d per head of high linoleic (n-6) cracked safflower seed (n = 32). Prior to initial study, heifers were allocated to one of two feedlot pens. Heifers were allowed ad libitum access to fresh water. Diets were fed twice daily at 0800 and 1600 for 30 d (Figure 3). Pre-experimental analysis determined that the

safflower seeds contained 17.1 % CP and 53% TDN and the barley grain contained 15.0% CP and 81% TDN on a DM basis. Safflower seeds were cracked with a hammer mill before mixing into the diet. The dietary amount of the safflower seeds were provided at levels similar to previously recorded by Scholljegerdes et al. (2004). Diets were not formulated to be isonitrogenous or isoenergetic, but did meet the nutritional requirements of a 12 mo and 396 kg of BW pubertal heifer (NRC, 2000).

Data Collection. Heifers were weighed individually at allocation, d 0 and 30 (Figure 3). Grab samples of diets were dried in forced- air oven for 24 h at 100° C using the AOAC (2000) procedure to determine the DM of each treatment diet.

Estrus and Pregnancy Detection. Each heifer was fitted with an Estrotect patch (Rockway Inc., Spring Valley, WI) to detect estrus during the 30 d treatment period and each day for 4 of the 40-d breeding period (Figure 3). The Estrotect patch is applied over the sacrum of the animal (located on the line between the hip bones and the tail head). The detector will remain white until it is triggered. Triggering takes place when the pressure from the brisket of a mounting animal turns the detector a bright pink. Estrotect patch color was recorded d 0, 30 and each day for 4 of the 40-d breeding period (Figure 3). Estrotect patches were replaced at d 30 of the trial and heifers were monitored for estrus activity for 4 d (d 34 to d 37) (Figure 3). All heifers received the 7-d CO-synch + CIDR insert and an injection of GnRH on d 42. The CIDR device was removed on d 49, followed by i.m. injection of $PGF_{2\alpha}$. Heifers were injected i.m. with GnRH and bred by TAI by a trained AI technician on d 51. The uterus of each heifer was examined by rectal palpation for pregnancy 40 d post breeding by licensed veterinarian.

Blood Sampling Procedures. Blood samples (~10 mL) were collected from one jugular vein of each heifer via venipuncture using 10-mL untreated Kendall Monoject Blood Collection (Tyco HealthCare Group LP, Mansfield, MA) tubes on d-0 of the experiment (Figure 3). Samples were allowed to clot at 4°C for 24 h then centrifuged at 1,850 x g for 30 min. Sera was harvested and stored at -20°C until assayed for P_4.

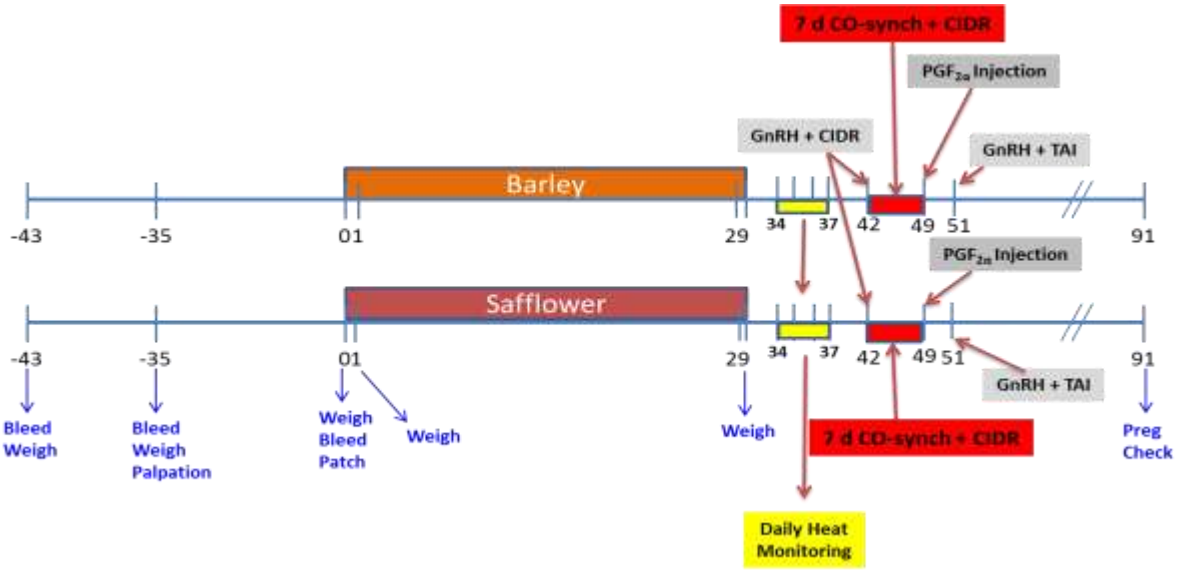

Figure 3. Timeline of samples and observations collected for both barley and safflower supplemented heifers at the NARC farm (Exp. 3).

Progesterone Assay. Progesterone concentrations were determined in serum samples in duplicate by a solid-phase RIA kit (Siemens Healthcare Diagnostics, Los Angeles, CA). The intra- and inter assay CV were 4.5 and 6.9%, respectively.

Statistical Analyses. Data for performance over the 30 d feeding period were statistically analyzed by ANOVA for a completely randomized design using the GLM

procedure of SAS (SAS Inst., Inc., Cary, NC). Least square means and associated standard errors are reported. Significance was set at an alpha level of 0.05.

Heifers were removed from summarized data if they were considered non-cyclic based on multiple progesterone concentrations < 1 ng/mL collected during the dietary treatment period (d 0 to d 30). Estrous responses for barley and safflower seed supplemented heifers that were observed twice daily for estrus from d 34 to d 37 after supplementation were analyzed separately by chi-square analysis using the PROC FREQ procedure of SAS. Pregnancy rates for barley and safflower seed supplemented heifers that were timed artificially insemination (TAI) after 7-d Control internal drug release (CIDR) protocol were analyzed by chi-square analysis using the PROC FREQ procedure of SAS (SAS Inst. Inc.). *P*-values were reported using Fisher's Exact Test, and significance was set at an alpha level of 0.05.

Results

Exp.1

No differences ($P > 0.05$) were observed between treatments for initial BW, final BW, live weight gain, ADG, or daily DMI (Table 3).

Table 3. Least square means for initial BW, BW gain, and DM intake for heifers supplemented with barley or safflower at BART[1] farm in Exp. 1

	Treatment			*P*-value
Item	Barley	Safflower	SEM	
n	23[2]	24		
Initial BW, kg	382.2	383.1	5.52	0.901
Final BW, kg	387.6	383.5	5.58	0.605
Live wt gain, kg	3.9	0.3	1.59	0.112
ADG, kg/d	0.13	0.01	0.051	0.112
DMI, kg/d	10.2	9.3	0.33	0.061

[1]BART = Bozeman Agricultural Research and Teaching Farm.
[2]One heifer was not present for a collection day.

Table 4. Estrus responses and pregnancy rates for barley and safflower supplemented heifers bred by AI during the 10-d breeding period (d 36 to d 41) at BART[1] farm in Exp.1

	Treatment		Chi-square	*P*-value
Item	Barley	Safflower		
n	24	24		
Estrous response, %	21	29	0.44	0.740
Pregnancy rate[2,3], %	40	73	5.14	0.036
Pregnancy rate[4], %	60	57	0.01	0.921

[1]BART = Bozeman Agricultural Research and Teaching Farm.
[2]Heifers diagnosed as anestrous or prepubertal during the treatment period by analyses of blood progesterone concentrations < 1 ng/mL were disregarded from this experiment.
[3]The overall percent pregnant of supplemented heifers for Exp.1.
[4]The percent pregnant of heifers bred AI 12 after onset of estrus from d 36 to d 41.

Table 5. Estrus response and pregnancy rate for barley and safflower supplemented heifers that did not display estrus by d 41 of the breeding period and were treated with 25 mg of $PGF_{2\alpha}$ and AI (d 41 to d 45) at BART[1] farm in Exp. 1

	Treatment		Chi-square	*P*-value
Item	Barley	Safflower		
n	19	17		
Estrous response, %	95	88	1.39	0.238
Pregnancy rate[2], %	42	71	2.95	0.107

[a]BART = Bozeman Agricultural Research and Teaching Farm. Heifers diagnosed as anestrous or prepubertal during the treatment period by analyses of blood progesterone concentrations < 1 ng/mL were disregarded from this experiment.
[b]The percent pregnant of heifers treated with 25 mg of $PGF_{2\alpha}$ and AI from d 41 to d 45.

Estrous response, after removal of the 35-d supplement (d 36 to d 41) did not differ between the barley or safflower treatments ($P > 0.10$) (Table 4). Differences in pregnancy rates between barley or safflower supplemented heifers bred AI 12 h after estrus were not detected (Table 4), as well as no differences in pregnancy rates between treatments in heifers that did not display estrus by d 41 and were injected with 25 mg of $PGF_{2\alpha}$ and AI by d 45 (Table 5). However, overall pregnancy rates was greater ($P < 0.05$) for safflower supplemented heifers than for heifers fed the barley supplement (Table 4). The complete reproductive results for Exp. 1 can be found in Appendix A.

There were no interactions between treatment and day for PGFM concentrations ($P > 0.05$). Concentrations of PGFM in pooled samples did not differ ($P > 0.05$) between barley and safflower treated heifers (Figure 4). However, mean PGFM concentrations differed ($P < 0.05$) between the highest mean PGFM concentrations at d 41 and d 34 and the lowest mean PGFM concentrations at d 2 and d 12 (figure not shown).

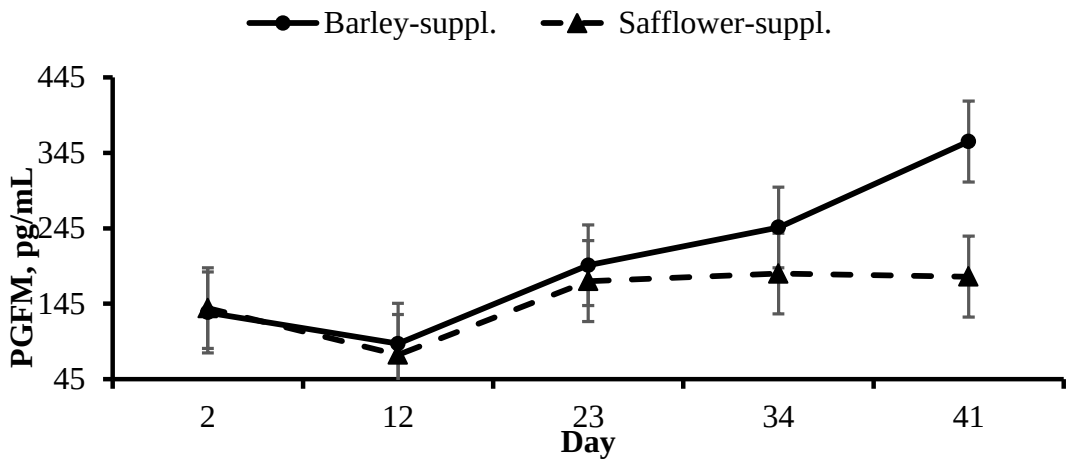

Figure 4. Least square means for pooled prostaglandin $F_{2\alpha}$ metabolites (PGFM) samples collected at d 2, 12, 23, 34, and 41 in heifers fed a barley or safflower supplement at the BART farm in Exp.1. Supplement type by d interaction, $P = 0.86$. Vertical bars represent SE for each mean.

Exp. 2

Heifers fed the barley supplement consumed more ($P < 0.001$) DM than heifers fed the high linoleic safflower diet (Table 6). However, there were no differences ($P > 0.05$) for initial BW, final BW, live weight gain, or ADG between the barley and safflower supplemented heifers (Table 6).

Table 6. Least square means for heifer initial BW, BW gain, ADG, and DM intake for barley and safflower diets at NARC[1] farm in Exp. 2

	Treatment			*P*-value
Item	Barley	Safflower	SEM	
n	20	20		
Initial BW, kg	402.0	390.9	7.27	0.288
Final BW, kg	417.4	407.4	7.72	0.368
Live wt gain, kg	15.3	16.4	1.86	0.675
ADG, kg/d	0.53	0.57	0.064	0.675
DMI, kg/d	10.8	8.9	0.20	<0.001

[1]NARC = Northern Agricultural Research Center.

Based on P_4 concentrations (< 1 ng/mL) measured over the feeding period, it was determine that 3 barley and 6 safflower heifers were anestrous and excluded from analyses. Estrous response, after removal of the 29 d supplement, (d 34 to d 37) did not differ ($P > 0.05$) between the barley or safflower treatments (Table 7). There were no differences ($P > 0.10$) between the barley and safflower treated heifers for pregnancy rates following AI 12 h after estrus (Table 7) or for heifers that did not display estrus by d 37 and received 25 mg of $PGF_{2\alpha}$ i.m. injection and TAI (Table 8). The complete reproductive results for Exp. 2 can be found in Appendix B.

Table 7. Estrous responses and pregnancy rates for barley and safflower supplemented heifers bred by AI (d 34 to d 37) at NARC[1] farm in Exp. 2

	Treatment		Chi-square	*P*-value
Item	Barley	Safflower		
n	17	14		
Estrous response, %	65	57	0.19	0.724
Pregnancy rate[2], %	41	21	1.37	0.280
Pregnancy rate[3], %	36	13	1.36	0.338

[1]NARC = Northern Agricultural Research Center. Heifers diagnosed as anestrous or prepubertal by analyses of blood progesterone concentrations < 1 ng/mL were removed from this experiment.
[2] The overall percent pregnant of supplemented heifers for Exp.2.
[3]The percent pregnant of heifers bred AI from d 34 to d 37.

Table 8. Estrous response and pregnancy rate for barley and safflower supplemented heifers that did not display estrus by d 37 of the breeding period and were treated with 25 mg of $PGF_{2\alpha}$ (d 37) and TAI (d 39) at NARC[1] farm in Exp. 2

	Treatment		Chi-square	*P*-value
Item	Barley	Safflower		
n	6	6		
Estrous response, %	100	100		
Pregnancy rate[2], %	50	33	0.34	0.558

[1] NARC = Northern Agricultural Research Center. Heifers diagnosed as anestrous or prepubertal by analyses of blood progesterone concentrations < 1 ng/mL were removed from this experiment.
[2]The percent pregnant of heifers treated with 25 mg of $PGF_{2\alpha}$ on d 37 and TAI on d 39

Exp. 3

Initial BW, final BW, live weight gain, and ADG did not differ ($P > 0.05$) between heifers fed the barley and safflower supplements in diets (Table 9).

Table 9. Least square means for heifer initial BW, BW gain, and ADG for heifers supplemented with barley or safflower at the NARC[1] farm in Exp. 3

	Treatment			*P*-value
Item	Barley	Safflower	SEM	
n	31	32		
Initial BW, kg	406.6	402.3	4.72	0.521
Final BW, kg	434.1	425.8	5.59	0.297
Live wt gain, kg	27.5	23.5	1.76	0.112
ADG, kg/d	0.92	0.78	0.059	0.115

[1]NARC = Northern Agricultural Research Center.

Based on P_4 concentrations (< 1 ng/mL) measured on d 0, 16 barley and 11 safflower heifers were assumed to be anestrous and excluded from analyses. The percentage of heifers that displayed estrus from d 34 to d 37 after the removal of safflower diet did not differ ($P > 0.05$) between the barley and safflower treatments (Table 10). There were no difference ($P > 0.05$) in conception rates between barley or safflower supplemented heifer diets (Table 10). The complete reproductive results for Exp. 3 can be found in Appendix C.

Table 10. Estrous response and conception rate for barley and safflower supplemented heifers observed from d 34 to d 37 and inserted with 7-d CIDR and TAI at the NARC[1] farm in Exp. 3

	Treatment		Chi-square	*P*-value
Item	Barley	Safflower		
n	15	21		
Estrous response, %	60	43	1.03	0.500
Conception rate , %	27	33	0.18	0.729

[1] NARC = Northern Agricultural Research Center. Heifers diagnosed as anestrous or prepubertal by analyses of blood progesterone concentrations < 1 ng/mL were removed from this experiment.

Discussion

Safflower seeds and percent of fat added were selected for these experiments based on previous experiments conducted by Bottger et al. (2002), Scholljgerdes et al. (2004), and Scholljegerdes et al. (2009).

Initial and final BW, live wt gain, ADG over the supplement period were similar between both the barley and safflower supplemented heifers. Our results are similar to those of Whitney et al. (2000) who reported no differences in initial and final BW or ADG in prepubertal beef heifers fed soybean oil. Individual intake was collected for both Exp. 1 and 2 with the GrowSafe system. In both experiments, DMI means appeared to be greater for heifers consuming the barley supplemented diet. Heifers fed the safflower supplemented diet consumed 82 % less (8.9 kg vs. 10.8 kg) than heifers fed the barley diet in Exp. 2. This difference in diet intake could be a result of the percentage of fat in safflower diet (3.8%) almost exceeding the recommended level of fat supplement > 4 % of DM (Hess et al., 2007), and therefore causing a decrease in forage digestibility (Whitney et al., 2000).

Although no experiments have been conducted in a similar fashion to our study, our observations were still comparable to numerous studies (Son et al., 1996; Filley et al. 2000; Alexander et al., 2002; Bottger et al., 2002; Lloyd et al., 2002; Hess. 2003;) that observed no difference between days to first estrus or pregnancy rate in heifers or cows supplemented with fat. The number of heifers that displayed estrus after the removal of the safflower diet was not influenced by feeding the high-linoleic safflower diet in this study. All the heifers on this study were in moderate body condition and had reached

puberty based on P4 concentrations > 1.0 ng/mL. The lack of a response between barley and safflower supplemented heifers probably is the result of analyzing only cyclic heifers for these experiments, and eventually these heifers would exhibit estrus during the observed time frame. However, visually comparing the percentages of heifers from both diet supplemented treatments that displayed estrous appeared to be greater for Exp.2 and 3 than those heifers in Exp. 1. A reason for the differences between experiments is not clear, and was not analyzed due to the difference in days estrus response was collected after the cessation of the treatment.

Pregnancy rates did not differ between barley and safflower supplemented heifers either bred by AI 12 h after the onset of estrus or treated with an i.m. injection of 25 mg of $PGF_{2\alpha}$ and either bred AI (Exp. 1) or TAI (Exp.2). Due to the low numbers of heifers that displayed estrus and bred AI 12 h in each experiment, we acknowledge the potential of type II errors, and a valid conclusion cannot be made for estrus response and pregnancy rate. However, we can conclude that overall pregnancy rates in Exp. 1 were affected by the high-linoleic safflower diet. Heifers supplemented with safflower in Exp. 1 were 82% more likely to be pregnant (9/23 vs 16/22) than the heifers fed the barley supplemented diet, no matter what AI protocol was used. Increased pregnancy rates could be the result of increasing the energy status, therefore, reserving more energy for reproductive functions (Hess, 2003). On the other hand, it could be possible the dietary fat in the safflower diet had an effect on hormone secretion to cause heifers in Exp. 1 to be more likely to be pregnant than the heifers fed the barley supplemented diet. It is known that P4 promotes maintenance of pregnancy and pubertal heifers supplemented

with fatty acids had increased concentrations of P4 that increased the chances of embryo survival (Lloyd et al., 2002). Yet, further studies using larger number of heifers are required to test whether pregnancy rate is affected by high-linoleic diets.

There were no differences in mean PGFM concentrations for the pooled serum samples between the barley and safflower supplemented heifers in Exp. 1. There are mixed views on whether a high linoleic diet increases (Filley et al., 2000; Hess, 2003) or inhibits prostaglandin synthesis (Lloyd et al., 2002), yet none comparable with Exp. 1 outcome. It is known that PGFM is used to measure the concentration of $PGF_{2\alpha}$ in circulation, which is involved in the regression of the corpus luteum. It could be possible that the pooled PGFM concentrations results are related to the lack of difference in estrus response between the barley and safflower supplemented heifers in Exp.1. On the other hand, significant differences in pooled PGFM concentrations were found between d 2 and 41, d 34 and 12, and d 41 and 12. It should be noted that the cessation of the supplement period was implemented on d 34. It is possible that more heifers had ovulated and reinitiated estrus by the end of the collection days, but individual PGFM concentrations would need to be examined to allow for more statistical power behind this idea. Nevertheless, we can conclude that concentrations of PGFM were not altered by feeding the high linoleic safflower diet during the 34-d supplement period.

In conclusion, supplementing beef heifers with high linoleic safflower seed appears to positively influence pregnancy rates, but can negatively affect DMI. However, the distribution of estrus after the removal of the fat supplement and the

temporal concentrations of PGFM during and after the removal of the fat supplement were not altered by high linoleic safflower seeds fed to pubertal beef heifers.

Implications

Supplementing replacement heifers with safflower seeds as a source of fat may increase overall pregnancy rates. It is important to note that increased supplemental fat may decrease feed intake especially if fat supplemented is over 4% of the total DM, and potentially negatively affect overall performance. Thus, it is important for cow-calf producers to determine if it is a logical strategy to add high linoleic fat to the diet to increase pregnancy rates in their replacement heifer herd.

Acknowledgements

Appreciation is expressed to Rockaway Inc. for donating all the Estrotect Heat Detectors used in this study.

CHAPTER 4

CONCLUSION

Cow-calf producers continue to struggle with improving reproductive efficiency in yearling beef heifers, resulting in more time and money being invested into developing and carrying them through calving. Adequate nutrition is vital for developing heifers and can be easily managed by cow-calf producers. Supplementing with fat is a fast and effective way to increase energy intake, satisfy maintenance demands, and potentially improving reproductive efficiency.

Supplementing with safflower seeds will affect DM intake, which can potentially harm overall heifer performance if not managed properly. Adding high linoleic safflower seeds to the diet does not appear to alter estrus response after the removal of the safflower seed, or PGFM concentrations during and after the removal of the supplement. However, supplementing with safflower seeds does appear to improve pregnancy rates in pubertal beef heifers.

Adding supplemental fats as a technique to manipulate reproductive functions in female cattle continues to draw attention, however more research is necessary with larger number of animals to obtain conclusive evidence that feeding feedstuff high in linoleic acid is beneficial in this regard.

LITERATURE CITED

Alexander, B. M., B. W. Hess, D. L. Hixon, B. L. Garrett, D. C. Rule, M. McFarland, J. D. Bottger, D. D. Simms, and G. E. Moss. 2002. Influence of prepartum fat supplementation on subsequent beef cow reproduction and calf performance. Prof. Anim. Sci. 18:351-357.

Anderson, L. H., C. M. McDowell, and M. L. Day. 1996. Progestin-induced puberty and secretion of luteinizing hormone in heifers. Bio. Reprod. 54:1025-1031.

AOAC. 2000. Official Methods of Analysis. 17th ed. AOAC, Arlington, VA.

Bellows, D. S., S. L. Ott, and R. A. Bellows. 2003. Review: Cost of reproductive diseases and conditions in cattle. Prof. Anim. Sci. 18:26-32.

Bottger, J. D., B. W. Hess, B. M. Alexander, D. L. Hixon, L. F. Woodard, R. N. Funston, D. M. Hallford, and G. E. Moss. 2002. Effects of supplementation with high linoleic or oleic cracked safflower seeds on postpartum reproduction and calf performance of primiparous beef heifers. J. Anim. Sci. 80: 2023-2030.

Bowman, J. G., and D. W. Sanson. 1996. Starch-or fiber-based energy supplements for grazing ruminants. In: Proc. West. Sect. Am. Soc. Anim. Sci. p 118-135.

Bridges, G. A., J. K. Ahola, C. Brauner, L. H. Cruppe, J. C. Currin, M. L. Day, P. J. Gunn. 2012. Determination of the appropriate delivery of prostaglandin F2α in the five-day CO-Synch + controlled intravaginal drug release protocol in suckled beef cows. J. Anim. Sci. 90:4814-4822.

Cheng, Z., R. S. Robinson, P. G. Pushpakumara, R. J. Mansbridge, and D. C. Wathes. 2001. Effect of dietary polyunsaturated fatty acids on uterine prostaglandin synthesis in the cow. J. Endocrinol. 171:463-473.

Dunn, T. G., and C. C. Kaltenbach. 1980. Nutrition and the postpartum interval of the ewe, sow and cow. J. Anim. Sci. 51: 29-39.

Dunn, T. G., and G. E. Moss. 1992. Effects of nutrient deficiencies and excesses on reproductive efficiency of livestock. J. Anim. Sci. 70: 1580-1593.

Field, T. G. 2007. Beef production and management decisions. 5th ed. Prentice Hall, Englewood Cliffs, NJ.

Filley, S. J., H. A. Turner, and F. Stormshak. 2000. Plasma fatty acids, prostaglandin F2alpha metabolite, and reproductive response in postpartum heifers fed rumen bypass fat. J. Anim. Sci. 78: 139-144.

Funston, R. N. 2004. Fat supplementation and reproduction in beef females. J. Anim. Sci. 82(E. Suppl.): E154–E161.

Galbreath, C. W., E. J. Scholljegerdes, G. P. Lardy, K. G. Odde, M. E. Wilson, J. W. Schroeder, K. A. Vonnahme. 2008. Effect of feeding flax or linseed meal on progesterone clearance rate in ovariectomized ewes. Domest. Anim. Endocrinol. 35:164-169.

Geary, T. W. 1997. Synchronization program update. In: Proc. Range Beef Cow Symp. XVI, Greeley, CO. pp 121-130.

Geary, T. W. 2003. Management of young cows for maximum reproductive performance. In: Proc. 35th Beef Improvement Federation Annual Meeting, Lexington, KY.

Grant, M. H. J., B. W. Hess, D. L. Hixon, E. A. Van Kirk, B. M. Alexander, T. M. Nett, and G. E. Moss. 2003. Effect of feeding high-linoleate safflower seeds on reproductive endocrine dynamics in postpartum beef females. Proc. West. Sec. Am. Soc. Anim. Sci. 54:36–39.

Grant, M. H. J., B. W. Hess, D.L. Hixon, E. A. Van Kirk, B.M. Alexander, T. M. Nett, and G. E. Moss. 2002 Influence of supplementation with safflower seeds on prostaglandin F metabolite in serum of postpartum beef cows. Proc. West. Sec. Amer. Soc. Anim. Sci. 53: 436-439.

Grummer, R. R., and D. J. Carroll. 1991. Effects of dietary fat on metabolic disorders and reproductive performance of dairy cattle. J. Anim. Sci. 69: 3838-3852.

Gulliver, C. E., M. A. Friend, B. J. King, and E. H. Clayton. 2012. The role of omega-3 polyunsaturated fatty acids in reproduction of sheep and cattle. Anim. Reprod. Sci. 131: 9-22.

Hall, J. B., A. Liles, and W. D. Whittier. 2009. Estrus Synchronization for Heifers. Publications & Resources.

Hess, B. W., D. C. Rule, and G. E. Moss. 2002. High fat supplements for reproducing beef cows: Have we discovered the magic bullet? Pages 59–83 in Proc. Pacific Northwest Anim. Nutr. Conf., Vancouver, British Columbia, Canada.

Hess, B. W. 2003. Supplementing fat to the cow herd. Pages 156–165 in Proc. Range Beef Cow Symp. XVIII, Mitchell. Nebraska Printworks, Scottsbluff, NE.

Hess, B. W., S. L. Lake, E. J. Scholljegerdes, T. R. Weston, V. Nayigihugu,, J. D. C. Molle, and G. E. Moss. 2005. Nutritional controls of beef cow reproduction. J. Anim. Sci. 83(E. Suppl.):E90–E106.

Hess, B. W., G. E. Moss, and D. C. Rule. 2008. A decade of developments in the area of fat supplementation research with beef cattle and sheep. J. Anim. Sci. 86: E188-E204.

Jaiswal, R. S., J. Singh, L. Marshall, and G. P. Adams. 2009. Repeatability of 2-wave and 3-wave patterns of ovarian follicular development during the bovine estrous cycle. Theriogenology 72:81-90.

Johnson, S. K., and R. Jones. 2005. Costs and comparisons of estrus synchronization systems. Proc. Appl. Reprod. Strategies Beef Cattle, Texas A&M University, College Station: 235-249.

Jones, B., R. D. Fish, A. Martin, G. C. Duff, and R. L. Ax. 2008. Case study: effects of supplemental linoleic and linolenic acids on reproduction in holstein cows. Prof. Anim. Sci. 24:500-505.

Juchem, S. O., R. L. A. Cerri, M. Villaseñor, K. N. Galvão, R. G. S. Bruno, H. M. Rutigliano, E. J. DePeters, F. T. Silvestre, W. W. Thatcher, and J. E. P. Santos. 2010. Supplementation with calcium salts of linoleic and -octadecenoic acids improves fertility of lactating dairy cows. Reprod. Dom. Anim. 45:55-62.

Lamb, G. C., J. E. Larson, T. W. Geary, J. S. Stevenson, S. K. Johnson, M. L. Day, R. P. Ansotegui, D. J. Kesler, J. M. DeJarnette, and D. G. Landblom. 2006. Synchronization of estrus and artificial insemination in replacement beef heifers using gonadotropin-releasing hormone, prostaglandin F2α, and progesterone. J. Anim. Sci. 84:3000-3009.

Lamb, G. C., J. S. Stevenson, D. J. Kesler, H. A. Garverick, D. R. Brown, and B. E. Salfen. 2001. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F2alpha for ovulation control in postpartum suckled beef cows. J. Anim. Sci. 79: 2253-9

Lands, W. E. 1992. Biochemistry and physiology of n-3 fatty acids. FASEB J. 6:2530-2536.

Lauderdale, J. W. 1974. Distribution and biological effects of prostaglandins. J. Anim. Sci. 38:22-30.

Lauderdale, J. W., B. E. Seguin, J. N. Stellflug, J. R. Chenault, W. W. Thatcher, C. K. Vincent, and A. F. Loyancano. 1974. Fertility of cattle following PGF2α injection. J. Anim. Sci. 38:964–967.

Lauderdale, J. W. 1975. The use of prostaglandin in cattle. Ann. Biol. Anim. Biochem. Biophys. 15: 419-425.

Lloyd, K. E., C. S. Whisnant, G. W. Huntington, and J. W. Spears. 2002. Effects of calcium salts of long-chain fatty acids on growth, reproductive performance, and hormonal and metabolite concentrations in pubertal beef heifers and postpartum cows. Prof. Anim. Sci. 18: 66-73.

Lucy, M. C. 2001. Reproductive loss in high-producing dairy cattle: where will it end? J. Dairy Sci. 84:1277-1293.

Lucy, M. C., J. D. Savio, L. Badinga, R. L. De La Sota, and W. W. Thatcher. 1992. Factors that affect ovarian follicular dynamics in cattle. J. Anim. Sci. 70:3615-3626.

Mallory, D. A., D. J. Wilson, D. C. Busch, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2010. Comparison of long-term progestin-based estrus synchronization protocols in beef heifers. J. Anim. Sci. 88:3568-3578.

Mattos, R., C. R., Staples, and W. W. Thatcher. 2000. Effects of dietary fatty acids on reproduction in ruminants. Reviews Reprod. 5:38-45.

NRC. 2000. Nutrient Requirements of Beef Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Odde, K. G. 1990. A review of synchronization of estrus in postpartum cattle. J. Anim. Sci. 68:817-830.

Patterson, D. J. J., B. Hall, N. W. Bradley, K. K. Schillo, B. L. Woods, and J. M. Kearnan. 1995. Improved synchrony, conception rate, and fecundity in postpartum suckled beef cows fed melengestrol acetate prior to prostaglandin F2 alpha. J. Anim. Sci. 73: 954-959.

Patterson, D. J., R. C. Perry, G. H. Kiracofe, R. A. Bellows, R. B. Staigmiller, and L. R. Corah. 1992. Management considerations in heifer development and puberty. J. Anim. Sci. 70:4018-4035.

Peel, R. K., J. L. Seabrook, G. E. Seidel, J. C. Whittier, A. V. Grove, and J. K. Ahola. 2013. Effect of gonadotropin-releasing hormone in long-term controlled

internal drug-release protocols on pregnancy rates in beef heifers artificially inseminated after observed estrus or fixed time. Prof. Anim. Sci. 29:228-236.

Pursley, J. R., M. R. Kosorok, and M. C. Wiltbank. 1997. Reproductive management of lactating dairy cows using synchronization of ovulation. J. Dairy Sci. 80:301–306.

Richards, M. W., J. C. Spitzer, and M. B. Warner. 1986. Effect of varying levels of post-partum nutrition and body condition at calving on subsequent reproductive performance in beef cattle. J. Anim. Sci. 62:300–306.

Robinson, R. S., P. G. Pushpakumara, Z. Cheng, A.R. Peters, D. R. Abayasekara, and D. C. Wathes. 2002. Effects of dietary polyunsaturated fatty acids on ovarian and uterine function in lactating dairy cows. Reproduction 124: 119-131.

Rodgers, J. C., S. L. Bird, J. E. Larson, N. DiLorenzo, C. R. Dahlen, A. Dicostanzo, and G. C. Lamb. 2012. An economic evaluation of estrous synchronization and timed artificial insemination in suckled beef cows. J. Anim. Sci. 90:4055-4062.

Scholljegerdes, E. J., B. W. Hess, G. E. Moss, D. L. Hixon, and D. C. Rule. 2004. Influence of supplemental cracked high-linoleate or high-oleate safflower seeds on site and extent of digestion in beef cattle. J. Anim. Sci. 82:3577-3588.

Scholljegerdes, E. J., B. W. Hess, M. H. J. Grant, S. L. Lake, B. M. Alexander, T. R. Weston, D. L. Hixon, E. A. Van Kirk, and G. E. Moss. 2009. Effects of feeding high-linoleate safflower seeds on postpartum reproduction in beef cows. J. Anim. Sci. 87: 2985-2995.

Short, R. E., and D. C. Adams. 1988. Nutritional and hormonal interrelationships in beef cattle reproduction. Can. J. Anim. Sci. 68:29–39.

Short, R. E., and R. A. Bellows. 1971. Relationships among weight gains, age at puberty and reproductive performance in heifers. J. Anim. Sci. 32:127-131.

Short, R. E., R. A. Bellows, R. B. Staigmiller, J. G. Berardinelli, and E. E. Custer. 1990. Physiological mechanisms controlling anestrus and infertility in postpartum beef cattle. J. Anim. Sci. 68:799-816.

Son, J., R. J. Grant, and L. L. Larson. 1996. Effects of tallow and escape protein on lactational and reproductive performance of dairy cows. J. Dairy Sci. 79:822-830.

Staples, C. R., J. M. Burke, and W. W. Thatcher. 1998. Influence of supplemental fats on reproductive tissues and performance of lactating cows. J. Dairy Sci. 81:856-871.

Talavera, F., C. S. Park, and G. L. Williams. 1985. Relationships among dietary lipid intake, serum cholesterol and ovarian function in holstein heifers. J. Anim. Sci. 60:1045-1051.

Tauck, S. A., J. R. C. Wilkinson, J. R. Olsen, J. N. Janitell, and J. G. Berardinelli. 2007. Comparison of controlled internal drug release device and melengestrol acetate as progestin sources in an estrous synchronization protocol for beef heifers. Theriogenology 68: 162–167

Thatcher, W. W., K. L. Macmillan, P. J. Hansen, and M. Drost. 1989. Concepts for regulation of corpus luteum function by the conceptus and ovarian follicles to improve fertility. Theriogenology 31:149-164.

Wathes, D. C., D. R. E. Abayasekara, and R. J. Aitken. 2007. Polyunsaturated fatty acids in male and female reproduction. Bio. Reprod. 77:190-201.

Whitney, M. B., B. W. Hess, L. A. Burgwald-Balstad, J. L. Sayer, C.M. Tsopito, C. T. Talbott, and D. M. Hallford. 2000. Effects of supplemental soybean oil level on in vitro digestion and performance of prepubertal beef heifers. J. Anim. Sci. 78:504-514.

Wiltbank, J. N., W. W. Rowden, J. E. Ingalls, K. E. Geegoey, and R. M. Koch. 1962. Effect of energy level on reproductive phenomena of mature hereford cows J. Anim. Sci. 21:219-225.

APPENDICES

APPENDIX A

COMPLETE REPRODUCTIVE DATA FOR EXP.1

Appendix A.-Estrus response and pregnancy rate for barley and safflower supplemented heifers at BART farm in Exp. 1

	Treatment		Chi-square	*P*-value
Item	Barley	Safflower		
n	24	24		
Overall pregnancy rate , %	37	71	5.37	0.042
Estrous response, %	21	29	0.44	0.740
n[1] (12h + AI)	5	7		
Pregnancy rate , %	60	57	0.01	0.921
n[2] ($PGF_{2\alpha}$ +AI)	19	17		
Estrous response, %	95	88	1.39	0.238
Pregnancy rate , %	42	71	2.95	0.107
n[3]	23	22		
Overall pregnancy rate[4] , %	39	73	5.14	0.036

[1]The number of heifers pregnant that displayed estrus and bred AI 12 h after (d 37 to d 41)

[2] The number of heifers pregnant that did not display estrus by d 41 and were injected with $PGF_{2\alpha}$ on d 41 and AI by d 45.

[3] Three heifers were excluded that did not show heat after injection of $PGF_{2\alpha}$ and were not AI.

[4] The percent of heifer pregnant excluding the three non-cyclic heifers.

APPENDIX B

COMPLETE REPRODUCTIVE DATA FOR EXP. 2

Appendix B.-Estrus response and pregnancy rate for barley and safflower supplemented heifers at NARC farm in Exp. 2.

	Treatment		Chi-square	*P*-value
Item	Barley	Safflower		
n	20	20		
Estrous response, %	60	50	0.40	0.751
Overall pregnancy rate , %	35	20	1.13	0.480
n[1]	17	14		
Estrous response, %	65	57	0.19	0.724
n[2] (12 h and AI)	11	8		
Pregnancy rate , %	36	13	1.36	0.338
n[3] ($PGF_{2\alpha}$ +AI)	6	6		
Estrous response, %	100	100		
Pregnancy rate, %	50	33	0.34	0.558

[1]The number of heifers deemed cycling based on analyses of blood progesterone concentrations < 1 ng/mL that displayed estrus and bred AI 12 h after (d 37 to d 41)

[2] The number of heifers pregnant that displayed estrus and bred AI 12 h after (d 37 to d 41)

[3]The number of heifers that were injected with $PGF_{2\alpha}$ on d 41 and AI by d 45.

APPENDIX C

COMPLETE REPRODUCTIVE DATA FOR EXP. 3

Appendix C.-Estrus response and pregnancy rate for barley and safflower supplemented heifers at NARC farm in Exp. 3.

	Treatment		Chi-square	*P*-value
Item	Barley	Safflower		
n	31	32		
Estrous response, %	39	44	0.16	0.799
n[1]	27	27		
Overall pregnancy rate , %	41	30	0.73	0.569
n[2]	15	21		
Estrous response, %	60	43	1.03	0.499
Pregnancy rate, %	27	33	0.18	0.729

[1]The number of heifers excluding heifers sold before determining pregnancy.

[2] The number of heifer's estrus response and pregnancy rates excluding anestrous and sold heifers. Heifers diagnosed as anestrous or prepubertal by analyses of blood progesterone concentrations < 1 ng/mL.