AN ABSTRACT OF THE THESIS OF

Emily C. Thompson for the degree of Master of Science in Animal Science

presented on October 12, 2012.

Title: Effects of α-linolenic and Linoleic Acids on Timed AI Reproductive Performance in Replacement Beef Heifers.

Abstract approved:

Chad J. Mueller

The objective of this study was to determine if supplementing linoleic or α-linolenic acids, and timing of supplementation around AI will alter supplemental intake, ADG, and serum progesterone (P_4), cholesterol, and triglyceride concentrations in replacement Angus crossbred heifers. In yr 1, 54 heifers, stratified by age, were assigned to: barley and soybean meal (CON); CON with 2% estimated DMI as flaxseed oil (F2); CON with 4% estimated DMI as flaxseed oil (F4); and CON with 4% estimated DMI as safflower oil (S4). In yr 2, timing of supplementation was evaluated on 51 heifers fed CON, F4 prior to AI (d 0) then CON (PREFLAX), CON prior to AI then F4 (POSTFLAX), or F4 throughout (FLAX). Isonitrogenous supplements were individually fed once per day and orts quantified. Supplement DMI (DMI_{SUPP}) was equal to 25% of estimated daily DMI (2.5% x BW). Grass hay and water were provided ad libitum. Heifers were estrous

synchronized using the 14-day CIDR-PG protocol. Data were analyzed as a completely randomized design using repeated measures with heifer as the experimental unit and heifer within diet as the error term. For yr 1, means were compared using the following contrasts: CON vs. OIL(F2, F4, S4), F2 vs. F4, and F4 vs. S4. Across mean comparisons were used in yr 2. No differences ($P > 0.10$) occurred in AI conception rates in either yr. Compared to CON, OIL had lower ($P < 0.05$) DMI_{SUPP}, but greater ($P < 0.05$) P_4 and cholesterol concentrations on d 22 and 29. Triglyceride concentrations were similar ($P > 0.10$) between CON and OIL during feeding. The F2 group had greater ($P < 0.01$) DMI_{SUPP} than F4, but there were no differences ($P > 0.10$) in P_4, cholesterol, and triglyceride concentrations. Supplemental DMI was lower ($P < 0.01$) for F4 versus S4, but no differences ($P > 0.10$) in P_4 concentration occurred. The S4 heifers had greater ($P < 0.05$) cholesterol concentrations on d 22 verses S4. In yr 2 PREFLAX and FLAX tended ($P < 0.10$) to have lower DMI_{SUPP} pre-AI, while FLAX tended to be lower ($P < 0.10$) post-AI. Progesterone concentrations were similar among supplements. The feeding of flaxseed oil resulted in greater cholesterol ($P < 0.05$) and triglyceride ($P < 0.10$) concentrations post-AI with POSTFLAX and FLAX heifers having greater concentrations than PREFLAX and CON on d 22 and 29. Feeding flaxseed oil around breeding has the potential to increase reproductive performance in beef heifers by increasing circulating progesterone and cholesterol.

KEY WORDS: polyunsaturated fatty acids, replacement beef heifer, timed AI

Effects of α-linolenic and Linoleic Acids on Timed AI Reproductive Performance in Replacement Beef Heifers

by
Emily C. Thompson

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented October 12, 2012
Commencement June 2013

Master of Science thesis of Emily C. Thompson presented on October 12, 2012

APPROVED:

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Emily C. Thompson, Author

ACKNOWLEDGEMENTS

This thesis is dedicated to my parents, Bob and Mary Thompson. Thank you for all the support you have given me throughout my college career. I cannot count the number of times I have called you guys, frustrated beyond belief with school and research, but you have always believed in me. You have pushed me to be the person I am today and I would not have made it without you. You have helped me both emotionally and financially, and have taken care of my critters when I could not take them with me. I am forever grateful.

I would also like to thank the rest of my family for all of the support you have given me through my seven long years of college. It often felt never ending, but everyone was always eager to hear the latest news at our family holidays. And a special thank you to my Grandma Thompson for all the help with textbooks; I am forever in your dept.

I would like to thank my committee members, Drs. Chad Mueller, Reinaldo Cooke, Tim DelCurto, and David Noakes for their patience. I know we have had to reschedule various meetings due to uncontrollable events, yet everyone remained so willing to work with me. Chad, thank you for continuing to encouraging me through all the chaos this last year; between family and personal health problems, you always understood. Thank you.

A special thanks goes to the farm crew (Kenny Fite, Mark Fite, Emery Gentry, and Jake Stockoff) for all their assistance in managing the cattle and helping during the numerous blood collections. Thanks to Grace Deboodt, Monique McKenzie, and Kelby Witherspoon for assistance with treatment administration and data collection. You three made my job much easier.

CONTRIBUTION OF AUTHORS

Thanks goes to Dr. Chad Mueller for assistance with data collection, data analysis, data interpretation, and design and writing of thesis; Dr. Reinaldo Cooke and Bruno Cappellozza for assistance with data analysis and data interpretation; and Dr. Tim DelCurto for assistance in design and writing of thesis.

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CHAPTER 1

Review of the Literature

UNITED STATES CONCEPTION RATES

Cattle ranchers in the U.S. have many hurdles to overcome in order to profit from their herd. Severe weather, grain prices, fuel prices, disease outbreaks, and predator attacks are all current issues that are faced in today's livestock industry. The primary goal of any cow-calf producer is to maximize the total number of calves born to a cow over her lifetime. According to the 2008 Beef Cattle Costs and Returns Study by the University of California Cooperative Extension, the estimated average cost, including feed, labor, and transportation a rancher invests a year is $575 per cow (Forero et al., 2008). It was estimated by Bellows et al. (2002) that $441 to $502 million is lost annually from beef cattle reproductive diseases and conditions that cause unsuccessful breeding or inability to maintain pregnancy. In order to maximize a cow's lifetime productivity, females must first calve at 2 years of age, and every year thereafter (Bagley, 1993). This means she must reach puberty no later than 12 to 13 months of age to conceive by the time she is 15 months. The more estrous cycles the heifer goes through prior to breeding, the greater her chances of conceiving early (Pond et al., 2005). It is estimated that a replacement heifer will not become profitable until she has weaned her fourth calf (Geary, 2003). It can be a challenge to rebreed two year old heifers because of the elongated period it often takes for young cows to return to estrus after their first

parturition (Geary, 2003). This postpartum anestrous can range 10 to 50 days longer for the first calf heifer than the average multiparous cow (Geary, 2003). A potential reason for the increase in postpartum anestrous is due to the additional demands placed on the young cow including continued growth and the stresses associated with first lactation (Spitzer et. al, 1995). Because of this delay in rebreeding, it is beneficial to breed yearling heifers as early as possible during their first breeding season.

A study by Bormann et al. (2006) estimated the average first service conception rates and pregnancy percentage of Angus heifers. Three thousand, one hundred and forty four replacement heifers from 6 herds located in 5 U.S. states (North Dakota, Kansas, Iowa, Oregon and Virginia) were utilized to determine both AI and overall pregnancy rates. Pregnancy was determined from 60 to 90 days post-breeding. First-service conception rate, which was defined as the percentage of heifers that became pregnant after the first AI service, was determined to be 60%. They also determined the pregnancy percentage, which was defined as the percentage of heifers pregnant at fall pregnancy check (conceiving via both AI and cover bull), at 93%. This data is similar to what the USDA reported in their 2007-08 Beef Cow-Calf Management Practices report, where 5.5% of heifers (± 1.2%) and 4.3% of cows (± 0.6%) failed to conceive after natural exposure or to AI. These studies provide insight into the average U.S. beef cattle conception rates for both heifers and cows, and provide ranchers a benchmark to compare the success of their own breeding programs.

Reproduction and the estrous cycle

The establishment of pregnancy is the result of a delicate balance of hormones generated during the appropriate time of the estrous cycle. The bovine estrous cycle consists of a 21 d cycle separated into two phases: the follicular phase and the luteal phase (Senger, 2003). The follicular phase occurs from the regression of the corpus luteum (CL) until ovulation occurs. During the follicular phase, the ovary starts producing estrogen for preparation of ovulation and mating (Senger, 2003). Once ovulation has occurred the luteal phase begins. This occurs when the follicular structure on the ovary transforms into the glandular CL (Figure 1; Senger, 2003). This structure secrets progesterone (P_4) and is responsible for the early maintenance of pregnancy prior to fetal recognition (Forde, 2011) from the embryonic production of interferon-tau (Senger, 2003). Progesterone is produced, at lesser amounts, from the adrenal glands (Asher et al., 1989), and from placental tissue during pregnancy (Shemesh, 1990). The progesterone from the CL prepares the uterus for implantation of an embryo (Funston, 2004), in addition to providing nourishment to the developing conceptus prior to implantation (Staples et al., 1998). Serum progesterone concentrations increase until around d 16 of the estrous cycle at which luteolysis, or CL regression, begins (Figure 2). If an embryo is not present or fetal recognition by the embryonic hormone interferon-tau is not present, an endometrial hormone called prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), which is inversely related to P_4 (Funston, 2004), is produced, causing the CL to regress (Senger, 2003). Prostaglandin $F_{2\alpha}$ is an important hormone necessary for the re-

establishment of estrous following parturition (Funston, 2004) and is detrimental to a newly developing embryo. Increasing progesterone concentrations around the time of AI have been associated with improved conception rates in ruminant animals (Funston, 2004). It is estimated that up to half of all mammalian embryos die during early gestation because of inadequate function of the luteal cells within the CL (Staples et al, 1998). Low progesterone concentration following breeding has been linked to reduced embryo development and increased $PGF_{2\alpha}$ synthesis during the critical period of maternal recognition (Wamsley et al., 2005).

Interferon-tau (IFNτ):

Interferon-tau (IFNτ) is an essential luteotrophic hormone produced during early gestation by the embryonic trophoblast cells and is highest between d 15 and 17 post-fertilization (Arosh, 2004; Senger, 2003). Interferon-tau is responsible for the delay in maternal oxytocin production and $PGF_{2\alpha}$ secretion from endometrial cells during early pregnancy, and in the absence of IFNτ the CL regresses and a new estrous cycle occurs (Senger, 2003). If IFNτ concentration is not elevated enough by d 17 for the maternal endometrial cells to recognize pregnancy, luteolysis will occur and result in embryonic death. Staples et al. (1998) estimated that the average early embryonic loss was 25 to 55%. Arosh et al. (2004) suggests that an inadequate response of the endometrium to IFNτ is likely to be a major reason for improper production of $PGF_{2\alpha}$ synthesis, and therefore pregnancy failure in cattle. Interferon-tau prevents the production of endometrial $PGF_{2\alpha}$, and

therefore CL regression, by blocking the receptors on the endometrium that bind with ovarian oxytocin and initiate prostaglandin synthesis (Senger, 2003).

Use of oil supplementation to improve reproductive efficiency

With the knowledge that increased concentrations of progesterone may improve conception rates, it is beneficial to explore easily applied ways to increase progesterone around the time of conception through nutritional manipulation. Supplementing oils to cattle to improve reproductive performance has been recognized for some time, but has often been attributed to the increase in dietary energy because of the high caloric density of oil (Hess et al, 2005). Ambrose et al. (2006) investigated the effects of flaxseed and sunflower seeds on ovarian function, early embryo survival, and conception rates in lactating dairy cows. When cows were supplemented with 8.7% flaxseed or 9.0% sunflower seed (DM basis; rolled) in their TMR from d 55 (± 22 d) to d 32 post AI, they found that cows fed flaxseed tended ($P < 0.07$) to have greater conception rates than those fed sunflower seeds (48.4% vs. 32.2%, respectively). Cows that received sunflower seeds tended to have a lower ($P = 0.08$) DMI and no differences ($P > 0.10$) occurred in plasma triglyceride, cholesterol and non-esterified fatty acids (NEFA) concentrations between the two treatments. Non-esterified fatty acids are reflective of the overall energy balance of the cow, and are lower when the animal is consuming a higher energy diet and therefore mobilizing less adipose tissue (Rabelo et al., 2005).

The maturation of follicles within the ovary is partially regulated by the overall energy status of the animal (Funston, 2004). When the energy status is at optimal levels, luteinizing hormone (LH) secretion from the pituitary occurs, causing ovulation (Funston, 2004). Without obtaining an optimal energy status, ovulation is unlikely to occur. The rate of body weight gain is the best indicator of the animal's nutritional status in young animals (Pond et al., 2005). Short and Bellows (1971) investigated the effect of rate of body weight gain on age of puberty in Angus x Hereford heifers. They used 3 treatment groups where heifers were supplemented with low, medium, or high feed levels starting at 7 months of age until approximately 13 months. Those on medium or high feed levels were given barley, linseed meal, wheat bran and molasses as mixed grain and diets were formulated to allow heifers to gain 0.28, 0.45, and 0.68 kg/d for low, medium, and high energy diets, respectively. They found that 83% of heifers who were supplemented with high gain diets had come into estrous prior to the breeding season while only 24% on the medium diet and 7% on the low diet attained estrous by the start of the breeding season.

Historically, dietary energy has been given credit for the increase in reproductive performance when lipid supplementation occurred, but in recent years, researchers such as Funston (2004) and Lopes, et al. (2009) have begun to question the contribution of dietary energy as the primary cause for increased reproductive performance in beef cows. The increase in reproductive performance seen when cattle are supplemented with lipids could be attributed to the formation of hormonal

precursors from polyunsaturated fatty acids (PUFA) in the diet (Wathes, 2007). These precursors allow for improved synthesis of hormones such as the steroidal hormone progesterone, from cholesterol, as well as the synthesis of prostaglandin $F_{2\alpha}$ from arachodonic acid synthesized from linoleic acids (Santos, 2008). Progesterone and $PGF_{2\alpha}$ have been determined to play vital roles during early pregnancy and fetal recognition, without which fetal recognition and continuation of pregnancy would unlikely occur.

Incorporation of Fatty Acids into Metabolism

Once ingested by the ruminant, the long-chain polyunsaturated fatty acids undergo biohydrogenation within the rumen (Williams and Stanko, 2000). Biohydrogenation is the addition of hydrogen to unsaturated fatty acids by rumen microbes (Church, 1988). Biohydrogenation of linoleic and α-linolenic fatty acids, were determined to be more than 70% and 85%, respectively, when fed as unprotected oil sources (Juchem, 2007). Certain modifications can be utilized to minimize biohydrogenation within the rumen such as the use of formaldehyde-treated casein coating (Church, 1988), or the binding of calcium salts to the fatty acid (Jenkins et al., 2007). Biohydrogenation of PUFA's requires multiple species of rumen bacteria and no single species is able to completely biohydrogenate the fatty acid without the assistance of another (Bauman et al.,1999). Daley et al. (2010) stated the microbial biohydrogenation of both linoleic and α-linolenic acid

are highly dependent on rumen pH, and when grain consumption increases, the bacterial biohydrogenation activity decreases due to a lowering of rumen pH.

Once biohydrogenated, lipids are transported through the digestive tract where they are absorbed at the proximal small intestine (Church, 1988; Hess et al., 2008). Figure 3 depicts the transformation of linoleic and α-linolenic acid from ingestion to hormonal production. Linoleic acid is converted to arachidonic acid (AA) by delta-6-desaturase and delta-5-desaturase by the addition of 1 or 2 double bonds in the fatty acid chain (Mattos et al., 2000), and is then serves as the substrate to 1- and 2-series prostaglandins (PG) within tissues throughout the body. Alpha-linolenic acid yields Eicosapentaenoic acid (EPA) which is converted to 3-series PGs (Wathes, 2007). Fatty acid desaturase 2 and 1 are both limiting within the liver, and PG production is affected by the type of PUFA by acting as a substrate for, or inhibitor of, cyclooxygenation. An increase of EPA from n-3 sources can depress metabolism of n-6 PUFAs and therefore the production of prostaglandins in the 2-series through these limiting pathways (Wathes, 2007).

Fatty acids, in addition to glycerides, are absorbed by cells and used to make energy and triglycerides within adipose and mammary tissue, or for steriodogenesis. Triglycerides are later used for a source of energy though it is not known if they play a role in ovarian function (Guedon et al., 1999). Triglycerides are free fatty acid triesters of glycerol and are the most abundant form of lipids (Voet and Voet, 1995). Concentrations of triglycerides are often greater when an

animal is consuming a low energy diet around calving and early lactation, or during periods of high energy demand and mobilizing adipose tissue (Rabelo et al., 2005). Rabelo et al. (2005) saw a 30% reduction in liver triglyceride concentration when dairy cows were fed high energy diets from time of calving until d 21 versus cows fed a low energy diet and attributed it to a low hepatic lipid metabolism.

Cholesterol

Cholesterol is the primary precursor for progesterone synthesis (Senger, 2003), and plasma cholesterol concentrations increase when supplemental fat is added to the diet of ruminants. Increased cholesterol concentrations are thought to increase steroidogenesis by the CL (Hawkins, 1995). In cattle, approximately 90 to 95% of the total cholesterol is in the form of HDL (Staples, 1998; Grummer and Carroll, 1991), and is thought to be the major plasma cholesterol source for steroidogenesis within bovine, porcine, and human follicular fluid (Grummer and Carroll, 1988). Once at the ovary, luteal cells incorporate the cholesterol and transport it into the inner mitochondrial membrane by the steroid acute regulator (STAR) protein (Wathes et al., 2000) where it is converted to pregnenolone. Pregnenolone then leaves the mitochondria and is enzymatically converted to progesterone (Senger, 2003) in the endoplasmic reticulum (Rekawiecki et al., 2008). Progesterone is then diffused out of the cell and travels to target tissues via the circulation (Senger, 2003).

A study by Hawkins et al. (1995) demonstrated that when beef heifers were fed a diet supplemented with Megalac, which consists of calcium soaps of long-chain fatty acids, serum concentrations of cholesterol and HDL were doubled along with an increase in progesterone and LDL. What was also interesting was the half life observed during their trial. Heifers fed Megalac had a significantly slower ($P = 0.02$) disappearance of serum progesterone after undergoing an ovariectomy. One of the conclusions from Hawkins et al., (1995) was that the increase of serum progesterone concentrations was not due to the increase in progesterone synthesis, but rather a decreased rate of clearance. This could be of importance because it could shorten the length of supplementation post-breeding while progesterone synthesis concentrations remain elevated

Fatty acids

The theory behind the addition of fatty acids, and therefore the increase in serum progesterone concentrations, is by either the increase in progesterone production or the decreased rate of clearance from the system (Williams and Stanko, 2000; Hawkins, 1995). In many studies, the idea is that the increased concentration of progesterone will elongate the time at which the luteal phase is dominant and therefore give additional time for the newly conceived embryo to produce the necessary hormones and proteins to establish pregnancy (Forde, 2011).

A study by Lopes et al. (2009) evaluated the effects of rumen-protected PUFA's on the reproductive performance of lactating *Bos indicus* beef cows. They

fed 0.1 kg/cow/day Megalac-E, a rumen protected source of linoleic acid, and supplemented from AI until 28 d post-breeding. They found that those supplemented with Megalac-E had greater ($P = 0.04$) pregnancy rates compared to control cows who did not receive the Megalac-E. This study also looked at the effects of Megalac-E on serum progesterone concentrations compared to cows supplemented with a rumen-protected saturated fatty acid, Megalac, fed at the same concentration of 0.1 kg/cow/day. Cows were supplemented from AI until 28 days post-breeding. They found that there were no differences ($P = 0.86$) in serum progesterone concentration, but cows who were supplemented with Megalac-E had a greater ($P = 0.02$) pregnancy rate compared to those supplemented with Megalac.

Previous research has given insight into the positive effects associated with rumen protected sources of PUFA's in both beef and dairy cows, but little research has been found pertaining to the reproductive performance of replacement beef heifers fed unprotected oil sources and their timing of supplementation in relation to AI. This research will look at the effects of two sources of oil, α-linolenic and linoleic acids, and the timing of supplementation, on the reproductive and hormonal response of replacement beef heifers.

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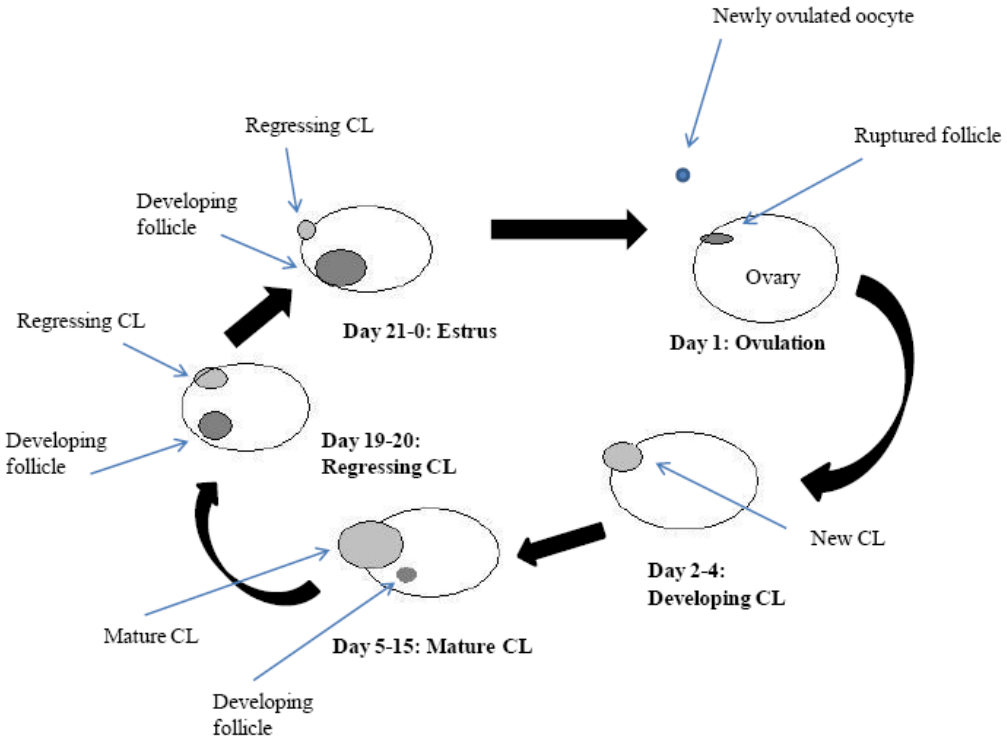

Figure 1: Bovine ovary during the estrous cycle. Adapted from Mathis and Parker (2006).

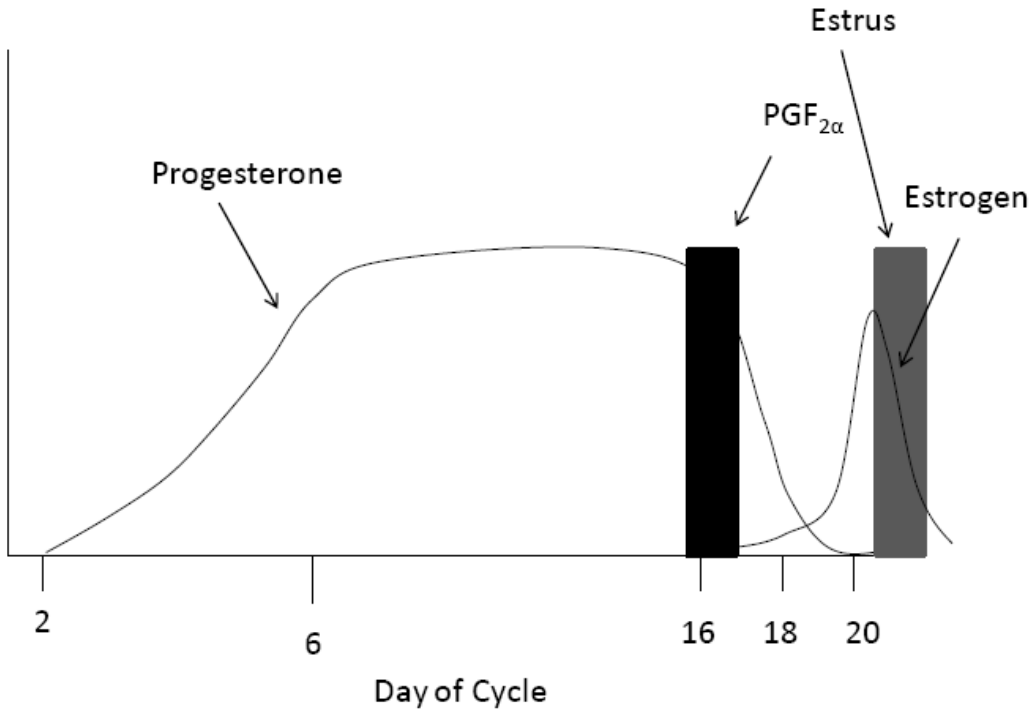

Figure 2: Bovine Estrous Cycle. Adapted from Mathis and Parker (2006).

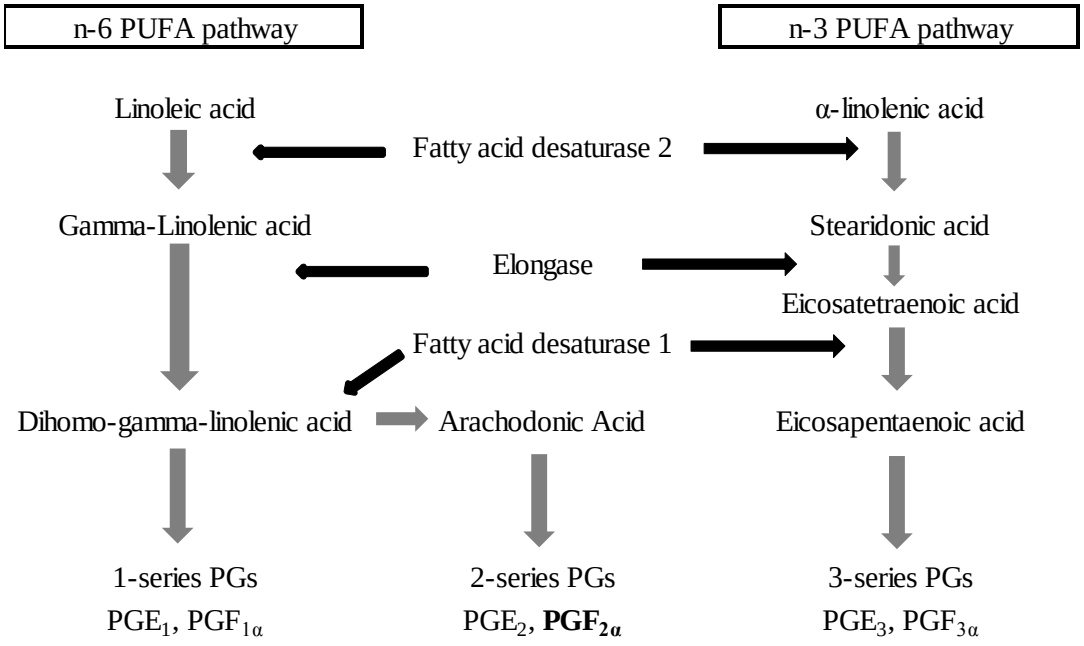

Figure 3: Fatty acid to hormone production. Adapted from Gulliver et al. (2012).

CHAPTER 2

Effects of dietary α-linolenic and linoleic acids on timed AI reproductive performance in replacement beef heifers

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ABSTRACT: The objective of this study was to determine if supplementing linoleic or α-linolenic acids prior to and after AI (TAI; d 0) will alter supplement intake, progesterone (P_4), cholesterol, or triglyceride concentrations in replacement beef heifers. Fifty four Angus crossbred heifers (age 381 ± 10.2 d) were stratified by age then assigned to one of four supplement groups: barley and soybean meal (CON); CON with 2% of DMI as flaxseed oil (F2); CON with 4% of DMI as flaxseed oil (F4); or CON with 4% of DMI as safflower oil (S4). Supplements were formulated to be isonitrogenous (18.25% CP, DM basis) and fed individually once per day with orts quantified. Supplement DMI (DMI_{SUPP}) was equal to 25% of estimated daily DMI (2.5% x BW). Grass hay and water were provided ad libitum. Heifers were estrus synchronized using the 14-day CIDR®-PG protocol. Blood was collected on d -36, 7, 15, 22, and 29 and serum utilized. Data was analyzed as a completely randomized design with repeated measures using the following contrasts: CON vs. OIL (F2, F4, S4), F2 vs. F4, and F4 vs. S4. Compared to CON, OIL had lower ($P < 0.05$) DMI_{SUPP}, but greater P_4 and cholesterol concentrations on d 22 and 29. Triglyceride concentrations were similar ($P > 0.10$) between CON and OIL during feeding. The F2 group had greater ($P < 0.001$) DMI_{SUPP} than F4 from, but there were no differences ($P > 0.10$) in P_4, cholesterol and triglyceride concentrations. Supplemental DMI was lower ($P < 0.001$) for F4 versus S4, but no differences ($P > 0.10$) in P_4 concentrations. The S4 heifers had greater ($P < 0.05$) cholesterol concentrations on d 22 verses F4. Feeding oil around AI has the potential to increase reproductive performance by elevating cholesterol and

progesterone concentrations, but no differences in conception rate occurred during this study.

KEY WORDS: polyunsaturated fatty acids, replacement beef heifer, timed AI

Introduction

The primary goal of any beef cattle producer is to produce a calf from each cow every year, starting at 2 years of age. Feeding polyunsaturated fatty acids can increase reproduction rates in cattle by increasing the progesterone concentration in the blood (Santos et al., 2008). Progesterone from the cow's corpus luteum (CL) is necessary for maintenance of pregnancy before fetal tissues take over hormonal control. Alpha-linolenic acid is able to reduce prostaglandin $F_{2\alpha}$ (PG $F_{2\alpha}$) synthesis from ovarian and uterine endometrial cells (Mattos et al., 2000), preventing the CL from regressing and reducing loss of pregnancy.

Mattos et al. (2003) evaluated the effects of *in vivo* endometrial cells incubated in eicosapentaonoic acid (EPA), the precursor for prostaglandin 3-series and the product of α-linolenic elongation and desaturation, on $PGF_{2\alpha}$ synthesis. When incubated for 24 h in EPA, endometrial cells showed a significant ($P < 0.05$) decrease in $PGF_{2\alpha}$ synthesis. In contrasts, cells incubated in arachodonic acid (AA), the precursor for prostaglandin 2-series, tended to have a greater ($P = 0.1$) secretion of $PGF_{2\alpha}$. It is thought that the availability of α-linolenic acid could inhibit $PGF_{2\alpha}$

by EPA displacing AA (Mattos et al. 2003). This data supports the results found by Ambrose et al. (2006) in supplementing α-linolenic and linoleic sources to 121 lactating dairy cows 28 d prior to breeding and at least 8 weeks in duration. They found that cows supplemented with α-linolenic acid had greater ($P < 0.01$) conception rates on d 21 than those supplemented with linoleic acid.

The hypothesis of this study is α-linolenic acid supplementation prior to and after AI will increase serum progesterone and cholesterol concentrations. The objectives were to determine the impact of supplemental oil prior to and after AI on conception rates, supplemental DMI, serum progesterone, cholesterol, and triglyceride concentrations in replacement beef heifers.

Materials and Methods

All methods were approved through the Oregon State University's Animal Care and Use Committee. Fifty four Angus-crossbred heifers (351 ± 24 kg), were stratified by age (381 ± 10.2d) and then randomly assigned to one of the following dietary supplement groups: barley and soybean meal (CON; n = 14); CON with 2% of estimated DMI as flaxseed oil (α-linolenic acid; F2; n = 13); CON with 4% of estimated DMI as flaxseed oil (F4; n = 14); and CON with 4% of estimated DMI as safflower oil (linoleic acid; S4; n = 13). Supplements were formulated to be isonitrogenous (18.25% CP, DM basis, Table 1) and were fed 21 d prior to and 21d post AI (d 0). Grass hay, loose mineral, and water were provided ad libitum throughout the trial. Daily DMI was estimated as 2.5% of heifers start weight.

Heifers received 25% of their daily DMI as supplement throughout the feeding period, with d -25 through d -22 designated as acclimation period. Diets (Table 1) were mixed daily in 45 kg batches and samples were taken daily from mixed treatments and weekly feed composites analyzed for CP by combustion nitrogen (Leco Corporation, St. Joseph, MI) and DM (AOAC, 2010). Fatty acid content of oil sources were analyzed via commercial laboratory (Table 2; Diagnostic Center for Population and Animal Health, Michigan State University, Lansing, MI). All heifers were fed individually daily in 2.5 x 5 m pens starting at 0800 and were allowed adequate time to consume their ration of supplement, or until they showed signs of satiety. Heifers were randomly selected from holding pasture for individual daily feeding of supplement.

Body weights were collected during all blood collection and diets adjusted appropriately on d -1. Health was monitored daily and treatment administered if necessary. All heifers were estrus synchronized using the 14-day CIDR®-PG protocol (Beef Reproductive Task Force, approved 2011). On d -33, a CIDR (Pfizer Animal Health, New York, NY) is inserted and removed 14 days later. On d -3 heifers received a 5 ml intramuscular injection of $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health, New York, NY). Heifers were inseminated and received a 2 ml intramuscular injection of GnRH (Fertagyl, Merck Animal Health, Summit, NJ) 66 h (± 2 h) later. All heifers were inseminated with a single common Angus sire by a qualified AI technician. Ten days after AI, a cover bull was commingled with

heifers. Pregnancy was determined on d 60 by rectal ultrasound and reconfirmed at calving.

Blood Collection

Heifers were bled on d -50, -42, -36, 7 15, 22, and 29 for serum analysis. All samples were collected via the caudal vein and extracted into 10 ml evacuated tubes (Vacutainer, Becton Dickinson and Co., Franklin Lakes, NJ) with no additives. Samples were placed on ice until refrigeration at 4°C for 24 hours to allow clotting. Tubes were centrifuged at 2000 x *g* for 30 minutes at 5°C, and serum was harvested and frozen (-20 °C) until analysis. Serum from d -50, -42, and -36 were used to determine estrous cyclicity. Serum progesterone (P_4) concentrations were determined on d -50, -42, -36, 7, 15, 22, and 29 using the ELISA assay described by Galvão et al. (2004). Serum P_4 inter-assay CV was 2.8% and mean intra-assay CV was 2.9%. A serum P_4 reading of 1 ng/ml for two consecutive collections was necessary to determine cyclicity prior to initiation of trial. Serum cholesterol concentrations were determined on d -36, 22, and 29 using a commercial quantitative-enzymatic-colorimetric assay kit (No. 1010, StanBio Laboratories, Boerne, TX) adapted to a microtiter plate design. Cholesterol concentrations were read in mg/dL and inter-assay CV was 7.9% and mean intra-assay CV was 7.6%. For analysis of triglycerides, a quantitative-enzymatic-colorimetric assay kit (No. 2100, StanBio Laboratories, Boerne, TX) adapted to a microtiter plate design was used on serum samples from d -36, 22, and 29.

Triglyceride concentrations were read in mg/dL and inter-assay CV was 14.6% and mean intra-assay CV was 15.3%.

Statistical analysis

Data were analyzed as a completely randomized design using repeated measures with dietary supplement as main effects. Heifer was the experimental unit and heifer within diet the error term. Means were separated using the mixed procedures of SAS (SAS Institute Inc., Cary, NC), and analyzed using the following pre-planned contrasts: CON vs. OIL (F2, F4, and S4), F2 vs. F4, and F4 vs. S4. For serum progesterone, cholesterol, and triglyceride concentrations, only heifers who conceived via AI were used in analysis.

Results and Discussion

One heifer in the F2 group was excluded from the statistical analysis due to a jaw injury that impeded supplement consumption for approximately one week. Three heifers that were in standing heat were bred the evening before timed-AI.

Using data obtained at calving, no differences ($P > 0.10$) in TAI conception or overall conception rate occurred within the three contrasts: Oil vs. CON, F2 vs. F4, and F4 vs. S4 (Table 3).

Heifers receiving CON had greater ($P = 0.011$) DMI_{SUPP} than OIL heifers from d -22 to AI (d 0) and ($P < 0.001$) from d 1 to d 22 (Table 4). The depression in DMI_{SUPP} observed by OIL could be contributed by the depression in the F4 supplement group that translated into the OIL contrast. On d 7, OIL heifers tended

to have greater ($P = 0.088$) progesterone concentrations than CON. On d 22 and 29, OIL tended to or had greater ($P < 0.05$) serum progesterone concentrations than CON and there was no difference ($P = 0.1199$) in serum progesterone concentrations on d 15 (Figure 4). On d 22 and 29, OIL heifers had greater ($P < 0.01$; Table 5) cholesterol concentrations than those in CON. On d 22, no differences ($P = 0.72$) in serum triglyceride concentrations occurred between OIL and CON, and on d 29, OIL heifers had greater ($P < 0.011$) concentrations than CON (Table 5).

Heifers in the F2 group had greater ($P < 0.001$) DMI_{SUPP} than F4 heifers from d -22 to AI and ($P < 0.001$) from d 1 to d 21 (Table 4). There were no differences ($P > 0.10$) in serum P_4 concentration between F2 and F4 heifers on d 7, 15, 22, and 29 (Figure 1). On d 22 and 29, there were no differences ($P = 0.73$ and 0.20) in serum cholesterol concentrations between F2 and F4 heifers (Table 5). There were no differences ($P > 0.10$) in serum triglyceride concentrations between the F2 and F4 heifers throughout the trial (Table 5).

Supplemental DMI was lower ($P < 0.001$) for F4 heifers compared to S4 heifers from d -22 to d 21 (Table 4). Serum progesterone concentrations were not different ($P > 0.10$) throughout the trial between the F4 and S4 groups (Figure 4). On d 22 and 29, S4 heifers had greater ($P = 0.036$ and 0.049) serum cholesterol concentrations than F4 heifers (Table 2). On d 22, no differences ($P = 0.72$) in

serum triglyceride concentrations occurred between F4 and S4, and on d 29, S4 heifers tended to have greater ($P < 0.067$) concentrations than F4 (Table 5).

Time from AI to calving was measured to determine if there was a difference in gestation length (Table 3). No differences ($P > 0.10$) were observed within all contrasts.

The results obtained from this study gave insight to a possible negative feedback mechanism associated with the supplemental ingestion of high levels of α-linolenic acid. Heifers in the F4 group showed a depression in DMI_{SUPP} that neither the F2 nor the S4 group demonstrated. This could indicate that the quantity of oil fed in addition to the oil source, rather than one or the other, could play a role in intake regulation (Table 4).

Another result was that despite the oil source, heifers supplemented with oil had higher serum progesterone concentrations than those on the control diet (Figure 4). This could be attributed to the increase in cholesterol seen for OIL heifers. Since cholesterol is the precursor for steroidogenesis, including the synthesis of progesterone (Hawkins et al., 1995), the incorporation of lipids into the diet, no matter which polyunsaturated fatty acid source (PUFA), could be enough to increase serum progesterone concentrations. Feeding high lipid diets, including linoleic acid, increases serum cholesterol (Garcia et al., 2003) and this could be one cause for the increase in progesterone in the S4 group. The increase in circulating cholesterol concentrations could be overriding $PGF_{2\alpha}$ synthesis from endometrial

cells since cholesterol is the primary precursor for progesterone synthesis (Mattos et al., 2000), but it should be noted that $PGF_{2\alpha}$ was not analyzed in this study.

Further research should be conducted determine if a metabolic or hormonal feedback mechanism is associated with the depression in consumption observed for the high α-linolenic acid treatment. It would also be beneficial to research whether there is a different metabolic response to both α-linolenic and linoleic acids between replacement and multiparous beef cows.

Implications

This research gave insight to the benefits of supplemental oil fed around time of breeding. Supplementation of oil around time of breeding increased circulating progesterone and cholesterol, which are both necessary for the early maintenance of pregnancy.

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Table 1: Nutrient Analysis of dietary supplements fed to replacement beef heifers

	Dietary Supplement			
Item	CON[1]	F2[1]	F4[1]	S4[1]
Barley, %	83.7	73.4	63.5	63.5
Soybean Meal, %	16.3	18.7	20.7	20.7
Flaxseed Oil[2], %	0.0	7.9	15.8	0.0
Safflower Oil[2], %	0.0	0.0	0.0	15.8
Nutrient Analysis				
DM, %	90.6	91.2	91.8	91.8
CP, %	18.1	18.2	18.1	18.1
NDF[3]	18.2	16.4	14.6	14.6
ADF[3]	6.8	6.3	5.7	5.7
NEm (Mcal/cwt)[3]	96.3	105.8	115.3	115.3
NEg (Mcal/cwt)[3]	64.5	72.2	79.8	79.8

[1] Treatments include control (CON), Flaxseed oil fed at 2% daily DMI (F2), Flaxseed oil fed at 4 % daily DMI (F4), and Safflower oil fed at 4% daily DMI (S4).

[2] % DM and % CP are determined via laboratory analysis. Flaxseed oil and safflower oil determined by Diagnostic Center for Population and Animal Health, Michigan State University, Lansing, MI based on laboratory analysis and inclusion rate.

[3] % NDF, % ADF, NEm, NEg are estimated from NRC 1996.

Table 2: Fatty acid composition of flaxseed and safflower oil fed pre- and post-AI on reproductive performance in replacement beef heifers

	% Oil Source	
Fatty Acid Analysis[1]	Flaxseed	Safflower
Palmitic (C16:0)	5.2	7.2
Stearic (C18:0)	4.4	3.1
Oleic (C18:1n9c)	20.2	17.6
Linoleic (C18:2n6c)	16.7	68.6
α-Linolenic (C18:3n3c)	51.7	0.4

[1]Diagnostic Center for Population and Animal Health, Michigan State University, Lansing, MI based on laboratory analysis and inclusion rate.

Table 3: Conception rate (%) and mean gestation length (d) of heifers supplemented with α-linolenic and linoleic acid.

	Dietary Supplement					Contrasts (*P*-values)[2]		
	CON[1]	F2[1]	F4[1]	S4[1]	SEM	OIL vs. CON	F2 vs. F4	F4 vs. S4
	Conception rate					Overall Conception		
AI[3], n (%)	7 (50.0)	8 (66.7)	9 (64.3)	8 (61.5)	0.16	0.61	0.68	0.88
Bull[3], n (%)	7 (50.0)	4 (33.3)	4 (28.6)	4 (30.8)				
Open[3], n (%)	0 (0.0)	0 (0.0)	1 (7.1)	1 (8.7)				
	Mean Gestation Interval, d							
AI	273.7	274.9	276.9	276.8	5.50	0.57	0.93	0.54
Bull	299.6	310.5	318.0	300.3				

[1] Treatments include CON= barley and soybean meal (n=14), F2= CON with 2% flaxseed oil (n=12), F4= CON with 4% Flaxseed Oil (n=14), and S4= CON with 4% safflower oil (n=13). Gestation interval (d) was the difference between calving date and AI date.
[2] Contrasts include CON vs. OIL (F2, F4, and S4), F2 vs. F4, and F4 vs. S4.
[3] Pregnancy status determined via ultrasound on d 60 by a Veterinarian and confirmed at calving.

Table 4: Number of individuals, starting age and weight, supplemental DMI, and ADG of replacement heifers fed α-linolenic and linoleic acids.

	Treatment Groups					Contrasts		
Item	CON[1]	F2[1]	F4[1]	S4[1]	SEM	CON vs. OIL	F2 vs. F4	F4 vs. S4
N	14	12	14	13				
Start Age, d[2]	381	380	380	382	2.9	0.92	0.95	0.76
Start BW, kg[3]	351	353	354	355	7.3	0.81	0.91	0.94
ADG, kg/d[4]	0.54	0.66	0.54	0.61	0.040	0.25	0.073	0.24
Daily DMI_{SUPP}, kg/d								
Pre AI[5]	2.2	2.2	1.7	2.2	0.06	0.011	< 0.001	< 0.001
Post AI[6]	2.4	2.4	1.5	2.3	0.05	< 0.001	< 0.001	< 0.001

[1] Control (CON), flaxseed oil fed at 2% of daily DMI (F2), flaxseed oil fed at 4% daily DMI (F4), and safflower oil fed at 4% daily DMI (S4). Oil includes F2, F4, and S4 means.
[2] Average age (d) at first blood collection.
[3] Average weight (kg) at first blood collection.
[4] Average daily gain from d -36 through d 22.
[5] d -22 to d 0
[6] d 1 to d 21

Table 5: Effects of α-linolenic and linoleic acid on serum cholesterol and triglyceride concentrations in replacement beef heifers who conceived via AI (mg/dL).

Days to AI	Dietary Supplement				SEM	Contrasts (*P*-values)[2]		
	CON[1]	F2[1]	F4[1]	S4[1]		CON vs. Oil	F2 vs. F4	F4 vs. S4
	Cholesterol, mg/dL							
-36	101.5	108.5	105.3	118.5	8.25	0.36	0.78	0.25
21	105.2	159.8	164.1	191.3	9.01	< 0.001	0.73	0.036
28	106.6	122.7	137.4	160.4	8.12	0.005	0.20	0.049
	Triglycerides, mg/dL							
-36	21.4	27.0	27.4	24.7	2.54	0.11	0.91	0.46
21	25.1	25.8	23.6	23.5	2.00	0.72	0.43	0.98
28	24.5	31.0	34.1	42.8	3.26	0.011	0.50	0.067

[1] Control (CON), flaxseed oil fed at 2% of daily DMI (F2), flaxseed oil fed at 4% daily DMI (F4), and safflower oil fed at 4% daily DMI (S4). Oil includes F2, F4, and S4 means.

[2] Contrasts include CON vs. OIL(F2, F4, and S4), F2 vs. F4, and F4 vs. S4.

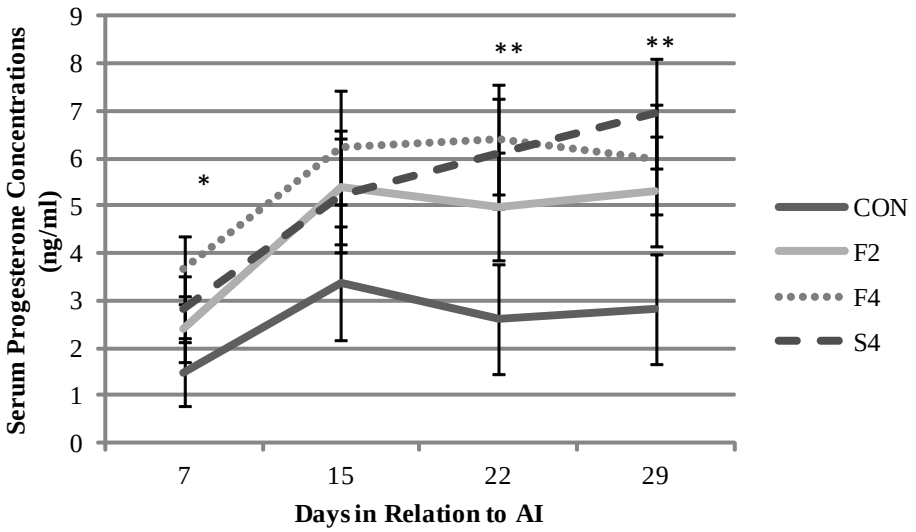

Figure 4: Effects of α-linolenic and linoleic acids on serum progesterone concentration in replacement beef heifers who conceived via AI. For the contrast CON vs. OIL, * $P < 0.10$ and ** $P < 0.05$. For the contrast F2 vs. F4, † $P < 0.10$ and †† $P < 0.05$. For the contrast F4 vs. S4, ‡ $P < 0.10$ and ‡‡ $P < 0.05$.

CHAPTER 3

Timing of dietary α-linolenic acid supplementation on AI reproductive performance in replacement beef heifers

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ABSTRACT: Fifty-one spring born Angus-crossbred replacement heifers were stratified by age (377 ± 12 d) and assigned to one of four supplement groups: barley and soybean meal (CON); CON prior to AI, then 4% flaxseed oil (POSTFLAX); 4% flaxseed oil prior to AI, then CON (PREFLAX); or 4% flaxseed oil throughout (FLAX). Supplements were formulated to be isonitrogenous (25% CP, DM basis) and heifers were individually fed from d -25 to 21 (AI; d 0). Supplement DMI (DMI_{SUPP}) was equal to 25% of estimated daily DMI (2.5% x BW). Grass hay, mineral mix, and water were provided ad libitum. Heifers were estrus synchronized using the 14-day CIDR-PG method. Blood was collected on d -36, 7, 15, 22, and 29 and serum analyzed. Means were separated and analyzed using a completely randomized design with repeated measures for progesterone, cholesterol, and triglyceride analysis. No differences ($P > 0.10$) in conception rates occurred. Heifers in CON and POSTFLAX had greater ($P < 0.05$) DMI_{SUPP} than PREFLAX and FLAX pre-AI. From d 1 to d 21, CON and PREFLAX had greater ($P < 0.05$) DMI_{SUPP} than FLAX, and POSTFLAX was not different ($P > 0.10$) from any other supplement groups. Progesterone concentrations were similar among supplements. The feeding of flaxseed oil resulted in greater cholesterol ($P < 0.05$) and triglyceride ($P < 0.10$) concentrations post-AI, with POSTFLAX and FLAX heifers having greater concentrations versus PREFLAX and CON on d 22 and 29. Supplementation of flaxseed oil after AI has the potential to increase reproductive performance by increasing circulating cholesterol.

KEY WORDS: α-linoleic acid, polyunsaturated fatty acids, replacement beef heifer, timed AI

Introduction

The primary goal of any beef cattle producer is to produce a calf from each cow every year, with her first parturition at 2 years of age. To ensure the female continues to produce a calf each year, it is beneficial that she conceives early during her first breeding season. This will allow her adequate time to return to estrus post-calving and prior to her second breeding season (Geary, 2003). It is known that feeding polyunsaturated fatty acids can increase reproduction rates in cattle by increasing the progesterone concentration in the blood (Santos et al., 2008). Progesterone from the cow's corpus luteum (CL) is necessary for maintenance of pregnancy before fetal tissues take over hormonal control. Alpha-linolenic acid is able to reduce prostaglandin $F_{2\alpha}$ (PG $F_{2\alpha}$) synthesis from endometrial cells (Mattos et al., 2000), preventing the CL from regressing and reducing loss of pregnancy.

Mattos et al. (2003) incubated endometrial cells for 24 hrs in eicosapentaonoic acid (EPA), the precursor for prostaglandin 3-series and the product of α-linolenic elongation and desaturation. They found that endometrial cells showed a significant ($P < 0.05$) decrease in $PGF_{2\alpha}$ synthesis. In contrast, cells incubated in arachodonic acid (AA), the precursor for prostaglandin 2-series, tended to have greater ($P = 0.1$) secretion of $PGF_{2\alpha}$. It is thought that the

availability of α-linolenic acid could inhibit $PGF_{2\alpha}$ by EPA displacing AA (Mattos et al. 2003).

The hypothesis of this research is flaxseed supplementation both one cycle prior to through one cycle post, and one cycle post AI will increase conception rates, and serum progesterone and cholesterol concentrations. The objectives were to determine if timing of supplemental flaxseed oil fed prior to and after AI affected conception rates, supplemental DMI; and serum progesterone, cholesterol, and triglyceride concentrations.

Materials and Methods

All methods were approved through Oregon State University's Animal Care and Use Committee. Fifty-one spring born, Angus cross-bred heifers (328 ± 29 kg) were stratified by age (377 ± 12 d) then assigned to one of the following dietary supplemental groups (Table 6): barley and soybean meal (CON, n = 12); CON prior to AI, then 4% flaxseed oil (POSTFLAX; n = 14); 4% flaxseed oil prior to AI, then CON (PREFLAX; n = 13); or 4% flaxseed oil throughout (FLAX; n = 12). Daily DMI was estimated as 2.5% of the heifer's individual BW, and heifers received 25% of their daily DMI as supplement. Diets were mixed daily in 45 kg batches. All heifers were moved to a holding pen daily then randomly selected for individual feeding in 2.5 x 5 m pens starting at 0800 and heifers were allowed adequate time to consume their ration of supplement, or until they showed signs of satiety. Fatty acid content of oil sources (Table 7) were analyzed prior to trial via

commercial laboratory (Diagnostic Center for Population and Animal Health, Michigan State University, Lansing, MI) and barley and soybean meal (SBM) were sampled weekly and composites analyzed for CP by combustion nitrogen (Leco Corporation, St. Joseph, MI) and DM (AOAC, 2010; Table 5). Dietary supplements were designed to be isonitrogenous (25%CP, DM basis).

Body weights were collected during all blood collection periods and supplement ration adjusted appropriately on d -1. Health was monitored daily and treatment administered if necessary using EOARC approved protocols. All heifers were estrus synchronized using the 14-day CIDR®-PG protocol (Beef Reproductive Task Force, approved 2011). On d- 33, a CIDR (Pfizer Animal Health, New York, NY) was inserted and removed 14 d later. On d -3 heifers received a 5 ml intramuscular injection of $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health, New York, NY). Heifers were inseminated and received a 2 ml intramuscular injection of GnRH (Fertagyl, Merck Animal Health, Summit, NJ) 66 h (± 2 h) later. All heifers were inseminated with a single common Angus sire by a qualified AI technician. Ten days after AI, a cover bull was commingled with heifers. Pregnancy was determined on d 64 by rectal ultrasound.

Blood Collection

Blood samples were collected from heifers on d -50, -42, -36, 7, 15, 22, and 29. All blood samples were collected via the caudal vein and extracted into a 10 ml evacuated tube (Vacutainer, Becton Dickinson and Co., Franklin Lakes, NJ) with

no additives. Samples were placed on ice until refrigeration at 4°C for 24 h to allow clotting. Tubes were centrifuged at 2000 x *g* for 30 min at 5°C, and serum was harvested and frozen (-20 °C) until analysis. Serum progesterone concentrations were determined on d -50, -42, -36, 7, 15, 22, and 29 using ELISA assay described by Galvão et al. (2004). The inter-assay CV was 4.8% and mean intra-assay CV was 4.8%. Serum progesterone from d -50, -42, and -36 were used to determine estrous cyclicity and a progesterone reading of 1 ng/ml for two consecutive collections was necessary to identify cyclicity. Serum cholesterol concentrations were determined on d -36, 7, 22, and 29 using a quantitative-enzymatic-colorimetric assay kit (No. 1010, StanBio Laboratories, Boerne, TX) adapted to a microtiter plate design. Cholesterol concentrations were read in mg/dL and inter-assay CV was 5.9% and mean intra-assay CV was 5.8%. For analysis of triglycerides, a quantitative-enzymatic-colorimetric assay kit (No. 2100, StanBio Laboratories, Boerne, TX) adapted to a microtiter plate design was used for serum samples from d -36, 7, 22, and 29. Triglyceride concentrations were read in mg/dL and inter-assay CV was 6.0% and mean intra-assay CV was 5.9%.

Statistical analysis

Main effects of supplementation time were analyzed as a completely randomized design using repeated measures and means were separated using mixed procedures (Proc Mixed, SAS Institute Inc., Cary, NC). Heifer was the experimental unit and heifer within diet was the error term. Serum progesterone,

cholesterol, and triglyceride concentrations were analyzed using repeated measure of heifers who conceived AI, due to the presence of a CL for all 4 blood collections post AI. Conception rates were analyzed using Chi-Square (SAS Institute Inc., Cary, NC).

Results and Discussion

No differences ($P > 0.10$) in overall conception rates were observed among mean comparisons (Table 8). Heifers in CON and POSTFLAX had greater ($P < 0.05$) DMI_{SUPP} than PREFLAX and FLAX heifers from d -22 to AI (Table 9). From d 1 to 21, CON and PREFLAX heifers had greater ($P < 0.05$) DMI_{SUPP} than FLAX, with DMI_{SUPP} POSTFLAX heifers not different ($P < 0.05$) from any other supplement groups (Table 9). No differences ($P > 0.10$) were detected for serum progesterone concentrations among mean comparisons on d 7, 15, 22, or 29 (Figure 5). On d -36, there were no differences ($P > 0.10$) in serum cholesterol and triglyceride concentrations across mean comparisons (Table 10). On d -36, POSTFLAX tended ($P = 0.095$) to have greater serum cholesterol concentrations than PREFLAX, and greater ($P = 0.036$) than FLAX. No other differences occurred on d -36. On d 7, CON heifers had lower ($P > 0.10$) serum cholesterol concentrations than FLAX and POSTFLAX heifers, and no difference ($P = 0.29$) than PREFLAX. Heifers in POSTFLAX had greater ($P = 0.003$) serum cholesterol concentrations than PREFLAX, and were not different ($P = 0.58$) than FLAX on d

7, and FLAX was greater ($P = 0.015$) than PREFLAX. On d 22, CON heifers had lower ($P < 0.05$) serum cholesterol concentrations than POSTFLAX and FLAX, and were not different ($P = 0.12$) than PREFLAX. On d 22, POSTFLAX had greater ($P < 0.001$) serum cholesterol concentrations than PREFLAX and tended ($P = 0.056$) to have greater concentrations than FLAX and, FLAX had greater ($P < 0.001$) than PREFLAX. On d 29, CON was not different ($P > 0.10$) than POSTFLAX and FLAX, and was greater ($P = 0.043$) than PREFLAX. On d 29, heifers in POSTFLAX had greater ($P = 0.001$) serum cholesterol concentrations than PREFLAX, and were not different than FLAX ($P = 0.20$), and FLAX was greater ($P = 0.030$) than PREFLAX (Table 10).

On d -36 there was no difference ($P > 0.10$) in serum triglyceride concentrations between treatment groups (Table 10). On d 7, FLAX tended ($P = 0.088$) to have greater triglyceride concentrations than CON. No other differences ($P > 0.10$) occurred on d 7. On d 22, serum triglyceride concentrations was not different ($P > 0.10$) between CON and PREFLAX, and POSTFLAX tended ($P = 0.100$) to be greater than CON, while FLAX was greater ($P = 0.039$) than CON. No other serum triglyceride concentration differences occurred ($P > 0.10$) on d 22. On d 29, POSTFLAX and FLAX had greater ($P = 0.001$ and 0.006) serum triglyceride concentrations than CON and no difference ($P = 0.17$) between CON and PREFLAX. Heifers in PREFLAX had lower ($P = 0.021$) serum triglyceride concentrations than PREFLAX and were not different ($P = 0.76$) than FLAX on d

29. Heifers in FLAX tended ($P = 0.071$) to have greater serum triglyceride concentrations than PREFLAX on d 29 (Table 10).

On d 22 and 29, FLAX and POSTFLAX heifers had greater ($P < 0.05$) serum cholesterol concentrations than PREFLAX and CON heifers. On d 7, FLAX and PREFLAX heifers had greater ($P < 0.05$) serum triglyceride concentrations than CON and POSTFLAX heifers (Table 10). On d 22, CON and PREFLAX heifers had lower ($P < 0.010$) serum triglyceride concentrations than FLAX and POSTFLAX heifers, and PREFLAX and FLAX heifers tended to be ($P = 0.099$) different. On d 29, FLAX and POSTFLAX heifers had greater ($P < 0.001$) serum triglyceride concentrations than CON and PREFLAX.

Since heifers consuming flaxseed oil after AI had a higher serum cholesterol concentration than those consuming flaxseed oil prior to AI, it would be beneficial to investigate further the duration of supplementation needed for the increase in cholesterol concentrations to occur since increased cholesterol has the potential to improve conception by elevating progesterone concentrations (Santos, 2008). Heifers fed flaxseed oil prior to AI had lower serum cholesterol concentrations by d 7 than those fed flaxseed oil throughout the trial, meaning the possibility for progesterone alteration was already greatly decreased before the crucial period of fetal recognition on d 15 through 17 (Arosh, 2004; Senger, 2003).

One possible reasons for the lack of difference in serum progesterone concentrations between the supplement groups is the decrease in DMI_{SUPP} seen

when heifers consumed the flaxseed oil supplement starting at the beginning of supplementation. Even though POSTFLAX heifers did not have a significant decrease in DMI_{SUPP}, those in PREFLAX and FLAX had lower DMI_{SUPP} while supplemented with flaxseed oil. It was observed that when heifers consumed flaxseed oil, they were less eager to consume the supplement and often took longer to do so than heifers consuming CON diets.

The results from this research showed there was little benefit of supplementing flaxseed oil only prior to AI. Supplementing flaxseed oil post AI seemed to have the greatest overall benefit because heifers consumed the supplement better than those fed flaxseed oil both pre and post AI and showed similar blood characteristics.

Implications

Results from this study give insight to the possible benefits associated with supplemental oil fed to replacement beef heifers. When heifers were supplemented with oil around the time of AI, serum cholesterol concentration increased. Since cholesterol is necessary for the synthesis of progesterone, a hormone necessary during early pregnancy, it is beneficial to increase circulating cholesterol around breeding.

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Table 6: Nutrient analysis of dietary supplements fed to replacement beef heifers.

	Dietary Supplement	
Item	CON	Flaxseed[1]
Barley, %	64.8	44.9
Soybean Meal, %	35.2	39.1
Flaxseed Oil[2], %	0.0	16.0
Nutrient Analysis		
DM, %	89.4	90.9
CP, %	25.1	25.0
α-Linolenic Acid[2], %	0.0	2.1
NDF[3]	16.1	12.5
ADF[3]	6.7	5.5
NEm (Mcal/cwt)[3]	96.6	115.9
NE (Mcal/cwt)[3]	65.2	80.6

[1] Flaxseed supplement applied to PREFLAX, POSTFLAX, and FLAX supplement groups.
[2] DM and % CP are determined via laboratory analysis. Diagnostic Center for Population and Animal Health, Michigan State University, Lansing, MI based on laboratory analysis and inclusion rate.
[3] % NDF, % ADF, NEm, and NEg are estimated from NRC 1996.

Table 7: Fatty acid composition of flaxseed oil fed at different times pre- and post-AI on reproductive performance in replacement beef heifers

Fatty acid composition, %[1]	Flaxseed oil
Palmitic (C16:0)	5.2
Stearic (C18:0)	4.4
Oleic (C18:1n9c)	20.2
Linoleic (C18:2n6c)	16.7
α-Linoleic (C18:3n3c)	51.7

[1]Analysis performed at the Diagnostic Center for Population and Animal Health, Michigan State University, Lansing, MI.

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Table 8: Conception rates (%) of replacement heifers supplemented with flaxseed oil at different times around AI.

	Dietary Supplement				
Item	CON[1]	POSTFLAX[1]	PREFLAX[1]	FLAX[1]	SEM
AI[2], n (%)	6 (50.0)[a]	9 (64.3)[a]	7 (53.9)[a]	7 (58.3)[a]	0.221[3]
Bull[2], n (%)	4 (33.3)	3 (21.4)	4 (30.8)	2 (16.7)	
Open[2], n (%)	2 (16.7)	2 (14.3)	2 (15.4)	3 (25.0)	

[a] $P > 0.10$

[1] CON= control fed -22 d through 21(n=12), POSTFLAX= supplemented CON d -22 through d 0 then fed flaxseed oil at 4% daily DMI from d 1 through d 21 (n=14), PREFLAX= treatment group fed flaxseed oil at 4% from d -22 through d 0 (n=13), then CON from d 1 through d 21. FLAX= treatment group fed flaxseed at 4% from d -22 through d 21 (n=12).

[2] Pregnancy statuses determined via ultrasound on d 64 by a Veterinarian.

[3] SEM determined using Chi Square analysis on overall conception.

Table 9: Starting age and weight, ADG, and supplemental DMI of heifers supplemented with α-linolenic acid at different times around AI.

	Treatment Groups				
Item	CON[1]	POSTFLAX[1]	PREFLAX[1]	FLAX[1]	SEM
N	12	14	13	12	
Start Age, d	390[a]	398[a]	396[a]	395[a]	3.4
Start BW, kg	325[a]	331[a]	329[a]	331[a]	8.3
ADG, kg/d	0.97[a]	0.99[a]	0.95[a]	0.90[a]	0.595
Daily DMI_{SUPP}, kg/d					
Pre AI[2]	1.8[a]	1.8[a]	1.3[b]	1.3[b]	0.07
Post AI[2]	2.0[a]	1.8[ab]	1.9[a]	1.5[b]	0.08

[a, b, c, d] Superscripts within row differ $P < 0.10$

[e, f, g, h] Superscripts within row differ $P < 0.05$

[1] CON= control fed -22 d through 21, POSTFLAX = supplemented CON d -22 through d 0 then fed flaxseed oil at 4% daily DMI from d 1 through d 21, PREFLAX = treatment group fed flaxseed oil at 4% from d -22 through d 0, then CON from d 1 through d 21. FLAX = treatment group fed flaxseed at 4% from d -22 through d 21.

[2] Supplementation from d -22 to d 0 (Pre AI), and supplementation from d 1 to d 21 (Post AI)

Table 10: Effects of timing of dietary flaxseed oil on serum cholesterol and triglyceride concentrations in replacement beef heifers who conceived via AI.

	Dietary Supplement				
Days to AI	CON[1]	POSTFLAX[1]	PREFLAX[1]	FLAX[1]	SEM
	Cholesterol mg/dL				
-36	139.2^{ab}	152.9^{be}	139.3^{a}	135.6^{af}	5.80
7	109.1^{e}	143.3^{fa}	118.2^{e}	139.1^{fa}	5.62
21	120.4^{ae}	169.1^{bf}	106.4^{ae}	153.5^{cf}	5.81
28	137.0^{ae}	151.8^{ae}	111.9^{f}	137.9^{ae}	7.88
	Triglycerides mg/dL				
-36	18.3^{a}	19.8^{a}	19.2^{a}	19.8^{a}	1.30
7	18.5^{a}	19.2^{a}	22.4^{ab}	23.1^{b}	1.80
21	14.4^{ae}	19.0^{b}	16.1^{ab}	18.2^{bf}	1.51
28	16.5^{ae}	21.5^{bf}	17.7^{ag}	21.4^{bfg}	1.19

$^{a, b, c, d}$ superscripts within row differ $P < 0.10$

$^{e, f, g, h}$ superscripts within row differ $P < 0.05$

[1] CON: barley and soybean meal fed -22 d to 21, POSTFLAX: supplemented CON d -22 to d 0 then fed flaxseed oil at 4% daily DMI from d 1 through d 21, PREFLAX: fed flaxseed oil at 4% from d -22 through d 0, then CON from d 1 to d 21, FLAX: fed flaxseed at 4% from d -22 to d 21.

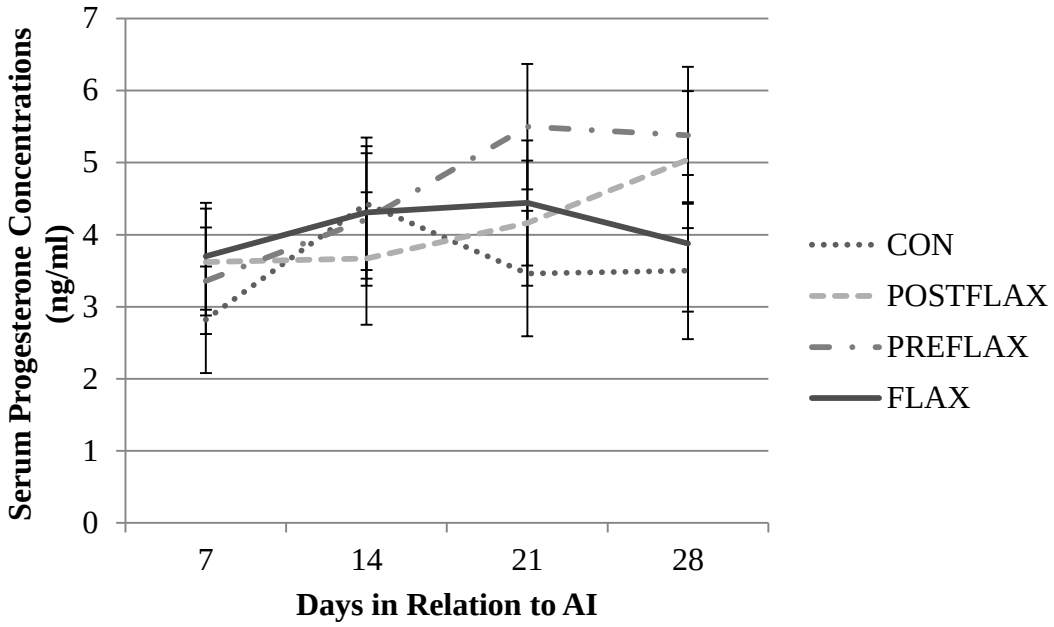

Figure 5: Effects of timing of α-linolenic acid on serum progesterone concentration around time of AI in beef replacement heifers who conceived via AI. *$P < 0.05$. CON= control fed -22 d through 21 (stippled), POSTFLAX= supplemented CON d -22 through d 0 then fed flaxseed oil at 4% daily DMI from d 1 through d 21 (long dash), PREFLAX= treatment group fed flaxseed oil at 4% from d -22 through d 0, then CON from d 1 through d 21 (short dash), FLAX= treatment group fed flaxseed at 4% from d -22 through d 21 (solid).

CHAPTER 4

The results obtained from both years of this project gave insight into a possible negative feedback mechanism associated with the supplemental ingestion of high levels of α-linolenic acid. During yr 1, heifers in the F4 group showed a depression in DMI_{SUPP} that neither the F2 group nor the S4 group showed. The incorporation of polyunsaturated fatty acids into the diet expresses more antimicrobial effects and inhibits more ruminal fermentation than saturated fatty acids (Hess et al., 2008) therefore decreasing the DMI of the animal. Scholljegerdes et al. (2011) evaluated the effects of short-term linolenic and linoleic supplementation on conception rates, estrous behavior and DMI. They found that when diets were formulated to provide 3.5% total dietary fatty acids, heifers that were supplemented with oil had lower ($P < 0.03$) forage intake than the control. In our study, intake was depressed in heifers receiving flaxseed oil at 4% DM, but not for heifers receiving safflower oil at 4%. This could indicate that the quantity of oil fed in addition to the oil source, rather than one or the other, could play a role in supplemental DMI.

During yr 1, heifers supplemented with either flaxseed oil at 4% or safflower oil at 4% both showed higher serum P_4 concentrations than those on the control diet. This could be attributed to the increase in cholesterol seen for OIL heifers. Since cholesterol is the precursor for steroidogenesis, including the synthesis of P_4 (Hawkins et al., 1995), the incorporation of lipids into the diet could

be enough to increase serum P_4 concentrations. Feeding of high lipid diets, including linoleic acid, increases serum cholesterol (Garcia et al., 2003) and this could be one cause for the increase in P_4 in the S4 group. The increase in circulating cholesterol could be overriding $PGF_{2\alpha}$ synthesis from endometrial cells since cholesterol is the primary precursor for progesterone synthesis (Mattos et al., 2000), though it should be noted that $PGF_{2\alpha}$ was not measured in this study.

Although yr 2 did not show an increase in serum progesterone concentrations like yr 1, data from this research shows that feeding α-linolenic has the potential to increase conception by increasing circulating cholesterol, which is needed for the production of progesterone (Santos, 2008). Since heifers fed flaxseed oil after AI had a higher serum cholesterol concentration than those fed flaxseed oil prior to AI, it would be beneficial to investigate further the duration of feeding necessary to achieve the elevated concentration.

One possible reason for the lack of difference in serum progesterone concentrations between the supplement groups is the decrease in supplemental DMI seen when heifers consumed the flaxseed oil ration. When heifers in yr 2 were fed the FLAX diet, which was the same as F4 in yr 1, their DMI_{SUPP} was lower than the other treatments for the respective year.

Cholesterol concentrations were similar to those seen by Guedon et al. (1999) when they looked at average cholesterol and triglyceride concentrations in beef cows. Triglyceride concentrations were slightly lower than those observed by

Guedon et al (1999), but Rabelo et al. (2005) stated that cows often have greater triglyceride concentrations due to the greater predisposition for fat mobilization from adipose tissue specifically after parturition.

Further research should be conducted to determine if a metabolic or hormonal feedback mechanism could be contributing to the depression in DMI consumption observed for the high α-linolenic acid treatment. It would also be beneficial to research whether there is a different metabolic response to both α-linolenic and linoleic acids between replacement and multiparous beef cows.

Implications

Although feeding both α-linolenic and linoleic acids to replacement beef heifers did not show an increase in conception rates, the supplementation of oil around time of breeding did show the possibilities for increasing reproductive performance by altering the cholesterol and progesterone concentrations within the animal. Since our study contained limited heifers, conception rate data were too limited. The diet that would be the most beneficial to a producer is the POSTFLAX fed during yr 2's research where 64% of heifers supplemented with flaxseed oil for 3 weeks post insemination conceived AI, while only 50% of heifers on the control diet conceived. While heifers from year 2 did not show a difference in progesterone concentrations from control, the heifers supplemented with the same diet (F4)

before and after AI the first year showed an increase in progesterone concentrations, and both years saw elevated cholesterol concentrations. Since POSTFLAX heifers were only supplemented after AI, the costs associated with acquisition, preparation, and distribution of supplemental flaxseed oil would be less than if supplemented before and after AI, and therefore more cost effective. More research should be conducted on the duration of supplementation necessary for the increase in serum cholesterol and progesterone observed in yr 1.

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Chapter 4

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