Effects of Feeding Supplemental Eicosapentanoic Acid and Docosahexanoic Acid to Beef Females on Reproductive Responses and Free Fatty Acids

by

Jeffrey Carl Wuenschel, Jr.

Thesis submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Animal and Poultry Sciences
(Physiology of Reproduction)

APPROVED

Dr. John B. Hall, Chairman

Dr. W.E. Beal

Dr. Scott P. Greiner

Dr. Katharine F. Knowlton

August 9, 2006
Blacksburg, Virginia

Key Words: Beef Cattle Reproduction Nutrition Prostaglandin Fatty Acids

Effects of Feeding Supplemental Eicosapentanoic Acid and Docosahexanoic Acid to Beef Females on Reproductive Responses and Free Fatty Acids

by

Jeffrey Carl Wuenschel, Jr.

ABSTRACT

The objective of this study was to determine the effects of dietary supplementation of eicosapentanoic (EPA) and docosahexanoic acids (DHA) on reproduction in beef females. In experiment 1, cows (n = 31) were individually fed rumen protected fish meal (FM) or no fish meal (C) supplements. Estrus was synchronized and ovulation induced on d 37. Ovarian follicular growth and diameter were determined by ultrasound on d 35 and d 37. Serum progesterone (P_4) profiles were analyzed on d 37 through d 52. On d 52 cows were cannulated, primed with estradiol-17β at -240 min, and stimulated to release $PGF_{2\alpha}$ by oxytocin injection at 0 min with blood sampled every 15 min from -30 min to 240 min. Supplement type did not affect ($P > 0.05$) follicular diameter, follicular growth or P_4 concentrations. In cows fed FM, prostaglandin metabolite (PGFM) concentrations tended ($P \leq 0.10$) to be reduced at 0, 30, and 60 min. In experiment 2, crossbred heifers (n = 214) received FM or C concentrates with corn silage from 30 d before estrous synchronization until 14 d after artificial insemination (AI). Serum fatty acid profiles were determined in five heifers from each group . Estrus detection and AI were conducted from d 37 through d 39. Dietary treatment increased ($P < 0.05$) EPA and DHA concentrations. Dietary treatment did not affect estrus response or AI conception rates and pregnancy rate. Supplementation of FM increased EPA and DHA concentrations but did not affect reproductive factors.

Key Words: Beef Cattle Reproduction Nutrition Prostaglandin Fatty Acids

DEDICATION

This work is dedicated to the present and future men and women of the United States armed forces and to the honorable memory of their predecessors. You have defended our shores against the enemy and planted freedom's flag in foreign lands giving hope to the oppressed and down trodden of this world. The citizens and leaders of this country little know how much we are in your debt. ***Semper Fidelis!***

We sleep safe in our beds because rough men stand ready in the night to visit violence on those who would do us harm. -*George Orwell*

There is a wonderful stone near the Tomb of the Unknown Soldier in Arlington Cemetery. It is dedicated to the Special Operations Forces. This marble stone, flush to the ground, sits under a large oak tree. Etched on this beautiful gray marble stone are the words from Isaiah 6:13, which read: "And the Lord said, who will go, who will fight for me," and the young Special Forces man stepped away from his family as they reached out, saying, "I will go, send me."

(Excerpt from *Hunting the Jackal* by Billy Waugh, 2004)

Acknowledgements

I owe a great debt of thanks to many people for their assistance over the past two years here at Virginia Tech. To those I don't list here I'm sorry if I have forgotten to thank you, but let it be known that I am grateful for your assistance.

Jehovah, I owe everything I am and ever will be to God. First, I thank you for the gift of salvation made freely available to me and all men through the death and resurrection of Jesus Christ. Second, thank you for always guiding me and knowing my path. Somehow, I always end up in the right place and things work out despite my plans. *Ut Deus solus exsisto gloria.*

Dr. John Hall, Thank you for taking me under your wing. Your time, effort, expertise, patience, and understanding have made the difference to me. Thank you for all the extra opportunities you have supplied to allow me to get "hands on" experience and for giving me the opportunity to find out that a state car will go 110 miles an hour. However, I value none of those as much as your friendship. For that I am truly grateful.

Mr. Lee Johnson, I'm sure I am only one in a long line of students and faculty who owe so much to you. I'm glad I'm leaving before you retire. I would have never survived in the lab without your expert guidance and patience. You have been the bottomless resource for all questions from computers to assays. I truly appreciate your assistance. I will miss your humor.

Dr. Bill Beal, Thank you for giving me the opportunity to work as your teaching assistant, for teaching me how to ultrasound, and for making your cows, farm, lab facilities, and personnel available to me for my research. Perhaps the most valuable thing you have shown me is your enthusiasm and professionalism in teaching. I have learned as much from you as any "intro" student. Should I teach some day, you have provided an excellent model for me to follow.

Mr. Henry Dickerson, You were invaluable help to me during my time at Kentland. Thank you for always going the extra mile for me.

Mr. Scott Purcell, Thank you for showing me the ropes when I first got here and letting me learn on your projects. You were the perfect graduate student and a darn hard act to follow when you left.

Ms. Brenda Caldwell, You have the patience of Job. Thank you for helping me so much and for your patience and expertise with the paperwork. Thank you the most though for caring.

Dr. Larry Kuehn, Without you I would be stuck in a *Dantesque* fifth ring of statistics. I don't know what the department and future graduate students will do without you.

Mrs. Wendy Wark and Dr. Joe Herbein, Thank you for running my fatty acid analysis. You saved me from a *Don Quixote* and the windmills like experience.

Dr. Carl Polan, Your advice and words of wisdom during my first semester are what kept me here. I would have never gotten to this point without you. Thank you.

The staff and work crews at the Virginia Department of Corrections Southampton Farm, I appreciate the loan of your heifers and for all the "hard time" you guys put in for me. We had a lot of good times. I will actually miss going to prison.

Doug Harris, Dave Whitlock, and the Select Sires Staff, Thanks for your help and expertise on my project, for teaching me the right way to AI, and for all the fun we had together. I now know what the difference between an "ooo" and an "ahh" is.

Animal and Poultry and Dairy Science graduate students, I would have never been able to pull my project off without your help. Too many of you to mention came out to Kentland. You have certainly made my life easier. Thank you also for all the questions you have patiently or not so patiently answered and for the fun times and companionship over the past years. A special thanks to Phoenix Rogers for not waking me up, to Kim Matson for taking care of me in bio-chemistry, and Stephanie Hill for not slapping me all those times she wanted to.

Jeff and Nancy Wuenschel Sr., Without you I wouldn't be here, literally. Thank you for giving me a good home, education, and love. Your support has always been the key to my success. I love you.

Mr. Gregory Stolfer and Mr. James Marsh, Thank you for introducing me to wrestling. You gave me a passion. The lessons I have learned on the mat have helped me to make it to this point. I will always be grateful for the opportunities and lessons you gave me both on and off the mat, for all the hard times, for all the good times, and for all the friends.

-It's not how much more you can take. It's how much more you can give.-

Special thanks to:

Select Sire Power, Inc., Thank you for providing the semen and logistical support for Experiment 2, conducted at the Southampton Correctional Institute.

Virtus Nutrition, LLC., Thank you for your generous donation of the EnerGII Reproduction Formula (now StrataG).

Table of Contents

List of Figures

List of Tables

Chapter 1: Introduction

Cattle play a multi-faceted role in the socio-economic fabric of the United States. They transform hundreds of thousands of acres of forages and tons of by-product materials into nutritious, palatable food and useful products for both the United States and the world. Cattle enterprises are present in all 50 states and produce the most popular protein consumed in the United States - beef (NCBA, 2005a). Considering the widespread nature of the industry and the popularity of its product with consumers, it is not surprising that the economic impact of the cattle industry in the United States is roughly $300 billion annually (NCBA, 2005b; Cryan, 2004). All this stems from approximately 42.7 million cows on less than one million farms with about 1.84% of the U.S. working population employed directly in the industry (NCBA, 2004; NCBA, 2005a; ERS, 2005).

Considering current and historic market trends of price fluctuation coupled with constant or increasing production costs, reproduction is vitally important to the success of the cattle industry. Reproductive performance is the most economically vital trait to beef cattle producers (Trenkle and Willman, 1977). Both efficiency of reproduction and opportunities to add value to the product can mean the difference between profit and loss. Artificial insemination (AI) allows producers to do both. Artificial insemination allows use of superior sires to increase the genetic merit of animals retained in the herd and those selected for harvest. The use of AI and estrus synchronization has been demonstrated to increase overall pregnancy rates by inducing cycles in anestrous cows and by increased presentation of animals for breeding (Sutphin, 2005). While utilization of AI may increase the average cost of a pregnancy, it is more than offset by the added

value to the producer at harvest. Calves from AI sires have been shown to require less days on feed while having a higher average daily gain and live weights at slaughter than non-AI sired calves (Sutphin, 2005). Artificially sired calves from AI sired dams returned an average of $135. 37 more per calf at slaughter to the producer than calves from non- AI sired dams bred by natural service sires (Sutphin, 2005). The increase in value is due to several factors, such as: greater breeding values of AI sires compared to natural service sires, increased age at weaning for AI sired calves, greater numbers of calves grading choice or better, higher percentages of calves with yield grades of 2 and 3, and decreased feed costs because of fewer days on feed and higher average daily gains.

Postpartum breeding often presents a significant challenge. Increased production traits such as calf size and growth and milk potential are negatively associated with reproductive efficiency (Jenkins and Ferrell, 2002). In addition, desirable carcass traits are negatively correlated with maternal traits (Greiner, 2001). Thus, producers are faced with the dilemma of how to maintain reproductive efficiency while selecting for desirable carcass and growth traits.

This thesis will review literature regarding the effect of fatty acid supplementation on metabolic status, ovarian follicular growth, and progesterone and prostaglandin dynamics as factors affecting reproductive efficiency. Furthermore, a two experiment study will be described, which was designed to test the hypothesis that in Experiment 1 supplementation of eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) would not affect release of $PGF_{2\alpha}$ after an oxytocin challenge, as measured by lower levels of the metabolite 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM), follicular diameter and development, or serum concentrations of progesterone. The hypothesis of Experiment 2

was that supplementation with EPA and DHA would not increase circulating levels of EPA and DHA or affect estrus response, conception rates, or pregnancy rates in heifers.

Chapter 2: Review of Literature

The Estrous Cycle

Puberty occurs in the female bovine between 9 and 24 months of age with the establishment of continuous estrous cycles. The bovine estrous cycle is approximately 21 days and is subdivided into the follicular and luteal phases. The follicular phase consists of proestrus and estrus; while the luteal phase is composed of metestrus and diestrus.

Estrus is the period of sexual receptivity and in the bovine lasts 6 to 24 hours. The dominant follicle secretes high levels of estrogen (E_2) during this period and late proestrus. Additionally,P_4 levels decrease due to the continued luteolysis of the corpus luteum (CL). These changes cause a surge of luteinizing hormone (LH) from the anterior pituitary (Senger, 1999) which triggers ovulation approximately 28 hours later. During the interval from the LH surge to ovulation a number of physiological changes take place in the pre-ovulatory follicle as a result of the spike in LH concentration. Follicular fluid volume and diameter increase, and the walls of the follicle become thinned and somewhat folded (Penderson, 1951; Priedkalns, et al., 1968). The number of gap junctions between the granulosa cells forming the walls of the antrum decrease as the wall degenerates and the granulosa cells become dispersed (Lipner, 1988; Murdoch and Cavender, 1987). Additionally, the oocyte and the cumulus oophorus become detached and float freely within the antrum (LeMarie, 1989). Concurrently, cells of the theca interna undergo hyperemia, edema, and erythrocyte effusion, possibly facilitating access of LH to the granulosa cells (Smith et al., 1994). Luteinizing hormone both blocks production of oocyte inhibiting factor and luteinizing inhibiting factor by the granulosa cells, as well as,

initiating synthesis of relaxin and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; Cunningham, 2002). The latter two substances promote formation of vesicles within the theca containing hydrolytic enzymes which breakdown the connective collagen matrix of the follicular wall (Cunningham, 2002). The combination of the above mentioned internal factors results in ovulation and the release of the oocyte into the oviduct.

Metestrus lasts 3 to 5 days and begins immediately upon ovulation and lasts until a fully functional CL is present (Senger, 1999). During this period both P_4 and E_2 levels are low due to immaturity of the forming CL and the newly recruited follicular wave. Cows tend to have two or three follicular waves (Lucy et al, 1992b), and unless ovulation occurs, all follicles undergo recruitment, selection, and atresia. Dominant follicles from the first and second waves become atretic due the presence of the CL on the ovary and the high secretion of P_4 which ameliorates LH secretion and prevents ovulation (Lucy et al., 1992b). The dominant follicle from the first wave becomes atretic during early diestrus thus removing E_2 and inhibin negative feedback on FSH. Thus, the process of recruitment, selection, dominance, and possible atrophy is started all over again. Note that in a mature cow, having three follicular waves, the second dominant follicle starts to become atretic at about the end of diestrus.

Following ovulation the follicle collapses within itself, resulting in inter-digitating folds of theca interna and granulosa cells which then begin to luteinize (Senger, 1999). The cells of the granulosa and theca interna undergo hypertrophy and hyperplasia to form large and small luteal cells respectively, although some transformation of small luteal cells to large has been reported (Smith et al, 1994; Fritz and Fitz, 1991). Signal amplification post-ovulation may be enhanced despite low hormonal levels due to an

increase in the number of inter-luteal cell gap junctions which facilitates transportation of intracellular messengers (Anderson and Little, 1984; Pate, 1996). Large luteal cells are responsible for the majority of P_4 secretion from the CL and exhibit greater intracellular levels of cytochrome P450scc enzyme than small luteal cells. Small luteal cells have been shown to be more dependent on LH, cAMP, and forskolin (Meidan et al., 1990; 1992). Concurrent with CL formation is a period of recruitment and selection of ovarian follicles known as folliculogenesis. Recruitment and growth through the primordial and primary stages is dependent on the availability of factors such as growth hormone and insulin. It is not until development reaches the secondary stage that the gonadotropin, follicle stimulating hormone (FSH), supplants them (Fortune, 2003; Hulshof et al., 1995). During early metestrus, several primordial follicles are recruited into a pool of growing follicles. The hypothalamus produces gonadotropin releasing hormone (GnRH), causing synthesis and release of FSH from the anterior pituitary. Follicle stimulating hormone is responsible for recruitment of follicles from the primordial stage and continued growth until the small antral stage is reached and selection occurs (Walters and Schallenberger, 1984; Lucy et al., 1992b). The mechanisms determining why one follicle is selected rather than any other follicle are as of yet unclear; however, at this stage the future dominant follicle becomes LH dependent (Ginther et al., 1996). At this point a fully functional CL is present and metestrus draws to a close as diestrus begins.

Diestrus stretches from the time luteinization is complete until luteolysis (Senger, 1999). Luteinizing hormone release during this phase is characterized by high amplitudes and low frequency. As the selected follicle continues to grow, it secretes E_2 and inhibin at an increasing rate, providing a negative feedback mechanism on both FSH

secretion and action (Padmanabhan et al, 1984; Savio et al., 1990). The unselected follicles then undergo atresia due to a lack of LH receptors.

Proestrus begins when luteolysis is initiated and ends when estrus begins (Senger, 1999). In cows having three follicular waves, the third follicular wave is recruited and begins the process toward domination at this time. In cows exhibiting 2 follicular waves, the dominant follicle continues development as the preovulatory follicle (Kastelic, 1994). Additionally, the CL begins to degenerate and loses its P_4 synthesizing ability in a process known as luteolysis which is primarily caused in the bovine by $PGF_{2\alpha}$ (Nancarrow et al., 1973; Senger, 1999). Degradation of CL tissue is known as structural luteolysis; while, loss of steroidogenic activity is referred to as functional luteolysis (Pate, 1994). Luteal synthesis of P_4 is diminished prior to the start of structural luteolysis (Sawyer et al, 1990). Estrogen from the developing dominant follicle activates oxytocin (OT) release from the posterior pituitary which in turn causes $PGF_{2\alpha}$ release from the uterus (McCracken et al., 1996; Homanics and Silvia, 1988). Estrogen also lowers the action potential of uterine smooth muscle tissue, rendering it more sensitive to oxytocin. Prostaglandin $F_{2\alpha}$ reaches the ovaries through a counter-current exchange system from the uterine vein to the ovarian artery (Senger, 1999) where it acts to both initiate luteolysis and up-regulate further OT and $PGF_{2\alpha}$ release from luteal and uterine sources (Niswender et al., 2000). Functional luteolysis begins primarily as a result of reduced blood flow to the CL through apoptosis of luteal tissues and corresponding diminutions in capillary density (Niswender et al., 2000). Prostaglandin $F_{2\alpha}$ also stimulates release of oxytocin from luteal cells and endothelin-1 from luteal endothelial cells. Thus, at early stages $PGF_{2\alpha}$ initiates a positive feedback mechanism on oxytocin and subsequently itself

(Schallenberger et al., 1984; Walters et al., 1984; Flint et al., 1990). Endothelin -1 acts to constrict ovarian capillaries and inhibit steroidogenic activity of luteal cells (Girsh et al, 1996). Prostaglandin $F_{2\alpha}$ binds to G-protein coupled receptors on the surface of the large luteal cells initiating a cascade of events leading to the release of intracellular Ca^{2+} which stimulates the action of the catalyst protein kinase C (Niswender et al., 2000). Protein kinase C in turn causes the modification of cellular proteins intrinsic to steroidogenesis, cholesterol availability, and maintenance of the luteal structural matrix (Niswender et al., 2000). Additionally, exposure to $PGF_{2\alpha}$ causes a reduction in the number and size of both large and small luteal cells (Braden et al, 1988). As blood flow is decreased and cellular machinery stopped or destroyed, there is a marked increase in ovarian populations of leucocytes, T-lymphocytes, and macrophages which facilitate apoptosis of the luteal tissues (Penny et al., 1999). As luteolysis progresses circulating P_4 is decreased, removing the negative feedback on the hypothalamus and anterior pituitary (McWilliams et al., 1998). Estrogen is secreted from the developing preovulatory follicle and signals an increase in the release of GnRH and LH, resulting in ovulation (McWilliams et al., 1998).

Maternal Recognition of Pregnancy

Should pregnancy occur, maintenance of the CL beyond the normal transitory pattern in the estrous cycle is necessary. Luteal P_4 plays an important role in ensuring nutrient availability to and survival of the conceptus through preparation of the uterine environment for pregnancy recognition, maintenance of uterine quiescence, and exertion of negative feedback on LH release (Inskeep, 2004). Bovine interferon τ (bIFN-τ) has

been widely accepted as the embryo produced signal which causes CL maintenance in early pregnancy (Senger et al, 1999; Thatcher et al., 1997; Roberts et al, 1992). Mononucleate cells of the bovine trophectoderm release bIFN-τ during blastocyst elongation (Roberts et al., 1996). Infusion of bIFN-τ has been shown to extend the duration of the estrous cycle and prolong luteal lifespan (Helmer et al., 1989; Knickerbocker et al., 1986a). Both amplitude and frequency of $PGF_{2\alpha}$ pulses are decreased by bIFN-τ, possibly through a reduction in uterine endometrial oxytocin receptors (Bazer et al., 1991; Knickerbocker et al., 1986b; Bazer et al., 1986). Mechanisms responsible for luteal maintenance beyond the first weeks of conception are unclear (Roberts et al., 1992). However, bIFN-τ appears to be the pivotal agent involved in maternal recognition of pregnancy.

Nutrition and Reproduction

Metabolic status has a significant impact on reproductive success in cattle (Randel, 1990; Hess et al., 2005). Of particular concern are developing heifers and postpartum cows which have higher energy requirements than other cattle. One of the main concerns affecting developing heifers is the onset of puberty. Puberty occurred earlier in heifers fed high energy diets than in heifers fed to gain only moderately or receiving no supplementation (Hall et al., 1995; Buskirk et al., 1996). Additionally, heifers fed high energy diets had greater body condition scores at puberty coupled with greater carcass weight and fat deposition, indicating the direct relationship between body composition and body condition score (Hall et al., 1995; Buskirk et al., 1996). Greater fat deposition indicates a greater level of energy availability which may be utilized for

reproduction. Inadequate nutrition results in metabolism of bodily stores and tissues for maintenance and production purposes at the expense of reproduction. Dunn (1980) reported 41% of embryos placed in first calf heifers which exhibited weight loss were lost as opposed to 24% for those heifers gaining weight.

Prepartum and postpartum nutrition restriction negatively affects the postpartum interval for both cows and first calf heifers (Randel, 1990). Nutrient restriction prepartum has also been associated with a decrease in dominant follicle size, delayed ovulation, increased failure to ovulate, and delayed resumption of normal luteal activity (Randel, 1990; Lalman et al., 1997; Mackey et al., 1999; de Vries and Veerkamp, 2000). Conversely, increased dry matter and energy intake has been shown to increase follicular recruitment and dominant follicle size, shorten periods of postpartum anestrous, and increase pregnancy rates (Randel, 1990; Gutierrez et al., 1997; Ciccioli et al., 2003).

Interest in fat supplementation began as a method of increasing dietary energy density in an effort to ameliorate negative energy balances in lactating postpartum cows (Hightshoe et al., 1991; Lucy et al., 1991a). However, several studies have indicated that positive increases in reproductive factors seen in cows fed various high fat diets may be due to the fatty acid composition of the diets rather than simply energy supplementation (Lucy et al., 1992; Petit et al., 2002). Lucy and coworkers, 1991b, fed postpartum lactating cows one of three diets: a control, a diet supplemented with calcium soaps of long chain fatty acids but with energy equivalent to the control, or a diet supplemented with calcium long chain fatty acids and having a higher energy value. Average size of preovulatory follicles was larger for cows fed the low energy diet supplemented with calcium soaps of long chain fatty acids than for the control or the high energy diet (Lucy

et al., 1991b). Other studies have also linked differences in reproductive effects of various fat sources to their fatty acid profiles (Petit et al., 2002; Staples et al., 1997), leading to an increased interest in fatty acids and their supplementation.

Fatty Acids

Fatty acids play an integral part in many biological activities. They store energy in the form of triacylglycerols, form cytoskeleton components and biologically isolated compartments, and act as intercellular and intracellular signals and hormone precursors (van Soest, 1994; Weng, 2002). Fatty acids are formed by the addition of 2 carbon units derived from acetyl-CoA which are broken down by the citric acid cycle for catabolism of metabolic fuel (Pratt and Cornely, 2004). While plants form fatty acids through photosynthesis, marine sources are supplied via consumption of phytoplanktons which also utilize photosynthetic pathways to synthesize fatty acids (Ching, 2000; Weng, 2002). Certain plants and plant derived substances, such as: soybeans, soybean oil, linseed, and flaxseed, are high in linoleic and linolenic acids; while marine sources typically express elevated levels of eicosapentanoic acid (EPA) and docosahexanoic acid (DHA; Weng, 2002).

Nomenclature of fatty acids is based on the number of carbons and double bonds in the individual acid structure. Saturated fatty acids have no double bonds; while those with one double bond are termed monounsaturated fatty acids. Those with two or more double bonds are called polyunsaturated fatty acids. Polyunsaturated fatty acids are named based on the position of the first double bond from the methyl end. For example: eicosapentanoic acid (C20:5 n-3) is a 20 carbon chain fatty acid having 5 double bonds,

the first one being located at the 3rd carbon from the methyl end. The methyl end is often referred to as the omega end; and n-3 and n-6 fatty acids are correspondingly referred to as omega-3 and omega-6 fatty acids respectively.

Eicosapentanoic acid and DHA (C22:6) are omega-3 polyunsaturated fatty acids which to some extent naturally escape rumen hydrogenation. Precursors to EPA and DHA, as well as, EPA and DHA themselves are often rumen protected through the calcium salts (soaps) process to ensure adequate levels escape rumen biohydrogenation and arrive intact in the gut for absorption. Examples of such are both the Megalac® (Church & Dwight Co., Princeton, NJ) and EnerGII® (Virtus Nutrition LLC, Fairlawn, OH) product lines which provide varying ratios of linoleic acid, linolenic acid, EPA, and DHA.

The precursor of EPA and DHA is linolenic acid (C18:3). The precursor of arachadonic acid (ARCH; C20:4 n-6) is linoleic acid (C18:2). Linolenic acid competes with linoleic acid for desaturation and elongation enzymes for synthesis of prostanoids and their precursors (Figure 2.1; Petit et al., 2002). Prostaglandin $F_{2\alpha}$ synthesis is rate limited by the availability of ARCH found in membrane phospholipids. Eicosapentanoic acid competes with ARCH for cyclooxygenase (COX) enzyme binding sites (Zhang, et al., 1995). Docosahexanoic acid is an inhibitor of COX (also known as prostaglandin endoperoxide synthase) activity. Displacement of ARCH by EPA results in the synthesis of series-3 prostanoids (i.e. PGI_3). Series 3 prostanoids are less biologically active and act on differing pathways than the metabolites of ARCH (series-2 prostanoids i.e. $PGF_{2\alpha}$ and PGE_2; Zhang et al., 1995). The series-3 prostanoids appear to have no role in bovine luteolysis (Thatcher et al., 2001).

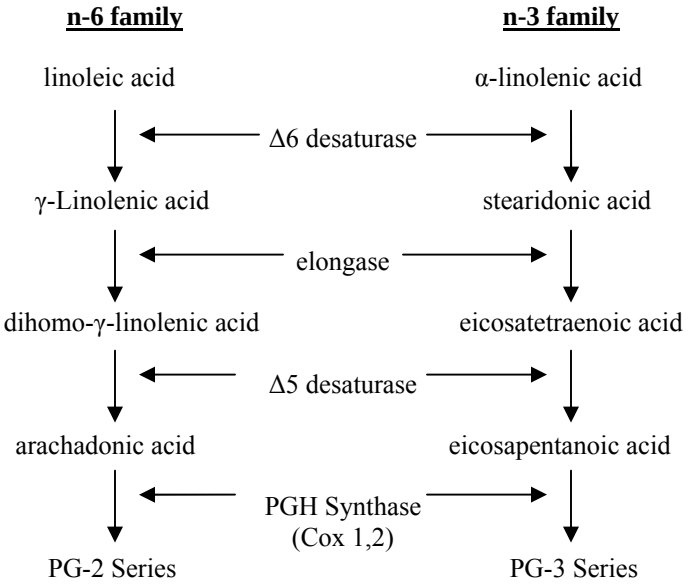

Figure 2.1. Precursors and enzymes leading to the formation of series 2 and 3 prostanoids.

In the ovine, it has been shown that as parturition approaches concentrations of ARCH increase in the uterine phospholipids - phosphatidylcholine, phosphatidylinosiol, and phosphatidylserine. This corresponds with a decrease in measurable concentrations of EPA in the same tissues (Zhang et al., 1995). The occurrence is not limited to ewes at parturition. Arachadonic acid concentrations in non-pregnant ewes peak on day 15 of the ovine estrous cycle in accordance with the initiation of structural luteolysis in addition to the functional luteolysis already underway (Meier et al., 1997).

Modification of uterine phospholipid fatty acid profiles may be achieved by supplementation of dietary fatty acids. In humans supplementation with EPA has been shown to decrease synthesis of ARCH metabolites (Barham et al., 2000). Fish oil supplementation in rat diets caused a diet dependent decrease in the ratio of ARCH to EPA (Howie et al., 1992). Burns and co-workers (2002, 2003) were able to increase plasma concentrations of EPA in primiparous cows by oral supplementation with

Menhaden fish meal. Additional data by Burns and co-workers (2000) reported an increase in subsequent relative concentrations of EPA and DHA in membrane phospholipids from bovine endometrial cells (BEND) cultured in medium supplemented with EPA and DHA. It has been suggested that these fatty acids inhibit $PGF_{2\alpha}$ synthesis, thereby decreasing early embryonic mortality (Thatcher et al., 2001).

Effects of Polyunsaturated Long Chain Fatty Acids on Reproductive Factors

The quantity of reported data available dealing exclusively with beef cattle is limited in reference to the effects of supplementing polyunsaturated long chain fatty acids on reproduction. However, a number of studies have been conducted and reported utilizing dairy cattle or cell cultures. These data have been presented in an effort to form a complete picture of the current state of knowledge in the subject. Table 2.1 has been included as a summary of relevant research. While direct application to beef cattle may not be advisable due to differences in intake and milk production, physiological systems are similar enough to warrant inclusion of the data.

Prostaglandin $F_{2\alpha}$. Several experiments have been conducted to determine the effects of EPA and DHA on $PGF_{2\alpha}$ secretion. Cultured bovine endometrial (BEND) cells were treated with EPA, DHA or linolenic acid and subsequently stimulated with phorbol ester to initiate $PGF_{2\alpha}$ secretion. Cells treated with EPA and DHA resulted in significant $PGF_{2\alpha}$ inhibition in a dose response manner compared to controls without fatty acid supplementation (Thatcher et al., 2001).

In vivo, up to 90% of $PGF_{2\alpha}$ is metabolized in one pass through the lungs (Cunningham, 2002). Therefore, the more stable prostaglandin metabolite 13,14-

dihydro-15-keto- $PGF_{2\alpha}$ (PGFM) is often used as a measure of how much $PGF_{2\alpha}$ has been present. Thatcher and co-workers (1997) fed estrous synchronized, lactating Holstein cows (n =15) iso-nitrogenous and iso-energetic diets differing only in the presence or absence of fish meal (FM). Injections of E_2 and OT were used to elicit release of $PGF_{2\alpha}$. Cows fed FM expressed decreased release of plasma PGFM (Figure 2.2; Thatcher et al., 1997).

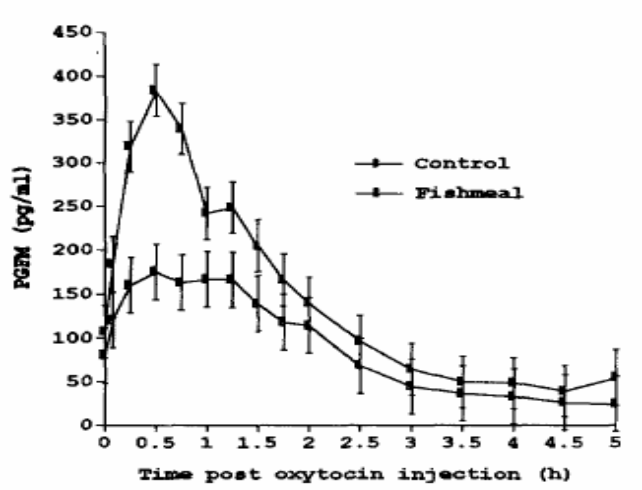

Figure 2.2. PGFM expression following induced $PGF_{2\alpha}$ with estradiol and oxytocin. Adapted from Thatcher et al. (1997).

In another study, lactating multiparous Holstein cows (n = 32) were fed four diets of varying FM concentrations (0, 2.6, 5.2, or 7.8% FM). The feeding of Menhaden FM reduced ($P < 0.05$) plasma concentrations of PGFM after E_2-OT challenge compared to the control. Additionally, the asymptotes of the PGFM regression curves calculated for cows from each of the three fish meal supplemented diets were more negatively sloped than those of control cows, possibly indicating not only lower plasma $PGF_{2\alpha}$ concentrations but also increased metabolism of $PGF_{2\alpha}$ (Mattos et al., 2002). Mattos and co-workers (2004) fed Holstein cows (n = 26) either fish oil or olive oil (C16:0, C18:1,

C18:2) supplemented diets beginning 21 days prior to their expected due date until 21 days postpartum. Fish oil treated cows exhibited decreased PGFM plasma concentrations during the period of maximal release in the early postpartum period. Differences ($P < 0.05$) were observed from day 0 through 2.5 postpartum. Convergence of plasma PGFM profiles by day 5 was attributed to shedding of caruncular tissue from the uterus which is largely responsible for $PGF_{2\alpha}$ synthesis. In contrast, minimal increases ($P < 0.07$) in PGFM concentration were reported for fish meal supplemented (50:50, FM: whole linseed) cows versus those on diets supplemented with Megalac, formaldehyde treated whole linseed, or duodenally infused linseed oil (Petit et al., 2002). However, the increase in PGFM concentration may be explained by the high omega-6 to omega-3 ratio in the fish oil treatment due to the inclusion of whole linseed. Similarly, Brahman cows supplemented with polyunsaturated fatty acids tended to exhibit an increase in peak PGFM concentrations (208.8 ± 47.2 pg/mL vs. 143.1 ± 50.1 pg/mL) compared to non supplemented cows (Lammoglia et al., 1997). Wamsley and co-workers (2005) reported that dietary supplementation with Menhaden fish meal only attenuated ($P < 0.05$) PGFM release after challenge with oxytocin in heifers with low luteal phase progesterone but otherwise had no effect.

Progesterone. Progesterone concentrations are often used as a measure of luteal function. Maintenance of high P_4 levels beyond a normal luteal life span seems to indicate reduced sensitivity of the CL to luteolysis. Increased P_4 concentrations during the luteal phase of the estrous cycle may be the result of increased synthesis.

Burke and co-workers (1997) supplemented diets of multiparous, lactating Holstein cows (n = 329) with either Menhaden fish meal or no supplement as control.

FM supplemented cows exhibited increased plasma progesterone profiles ($P < 0.01$) and a delayed response to exogenous $PGF_{2\alpha}$ ($P < 0.025$; Burke et al., 1997). After approximately 26 days of supplementation with linoleic and linolenic acid, multiparous, cyclic Brahman cows (n = 10) expressed increased ($P < 0.01$) serum P_4 concentrations beginning on day 5 of the estrous cycle versus control cows (Lammoglia et al., 1997). Multiparous Simmental cows (n = 12) receiving a Megalac supplemented diet exhibited greater concentrations of serum progesterone on days 6 and 8 ($P < 0.02$) of a synchronized estrous cycle than controls (Hightshoe et al., 1991). However, Wamsley and co-workers (2005) reported no effect of Menhaden fish meal supplementation on progesterone profiles of nulliparous beef heifers.

Ovarian Dynamics. Polyunsaturated fatty acids may also affect ovarian follicular growth. Supplementation of multiparous Holstein cows with fish meal increased the number of class II follicles and tended toward a greater diameter of the largest follicles. Cows receiving greater amounts of omega-3 fatty acids had the greatest average CL size (Petit et al., 2002). Supplementation with rice bran, a feed high in oleic and linoleic acid, in Brahman cows tended to increase ($P < 0.09$) the number of follicles during both the first and ovulatory follicular waves (Lammoglia, et al., 1997). Inclusion of Megalac in the diets of multiparous, lactating Holstein cows (n = 45) fed 15.7% degradable intake protein tended to increase number of CL, size of the largest CL, and total luteal area ($P = 0.074$, 0.088, and 0.101) when compared to cows receiving the same level of degradable protein intake without calcium salts of long chain fatty acid supplementation (Garcia-Bojalil, et al., 1998). Hightshoe et al (1991) reported that supplementation of Megalac to multiparous Simmental cows enhanced the growth of class two follicles (6 to 9 mm) into

both class three (10 to 15 mm) and class four (> 15 mm) follicles. Multiparous Holstein cows (n = 18) fed a diet supplemented with Megalac had a greater proportion of class 4 follicles, as well as, larger sizes of both the dominant ($P < 0.04$) and second largest follicle ($P < 0.07$) than cattle fed a control diet (Lucy et al., 1991a).

Interestingly, when cultured β-TC3 insulinoma cells from the islet of Langerhans in the pancreas are supplemented with EPA, insulin secretion is augmented (Konrad et al., 1996). Insulin is necessary for follicular activation and subsequent growth (Fortune, 2003). Insulin increased the percentage of healthy follicles and in combination with IGF-I and IGF-II increased the number of follicles in the primary stage (Louhio et al., 2000). Increases in plasma insulin levels ($P < 0.01$) have been associated with linoleic and linolenic acid supplementation (Lammoglia et al., 1997). Mature beef cows were supplemented with either animal tallow (saturated fat), soybean oil (polyunsaturated fat), or fish oil (highly polyunsaturated fat). Soybean oil in fat supplemented cows increased serum insulin more quickly than other treatments. However, by week 7 of supplementation serum insulin concentrations of all fat supplemented cattle were significantly increased over controls. Fat supplementation increased the number of medium size follicles (Thomas et al., 1997). The number of medium size follicles was greater in the group supplemented with soybean oil relative to the other groups (Thomas et al., 1997). Therefore, EPA may influence follicular growth indirectly through increased insulin concentrations.

Cholesterol, Insulin, Luteinizing Hormone, and Estrogen. Researchers have reported varied results concerning the effects of long chain fatty acid supplementation on concentrations of cholesterol, growth hormone, insulin, LH, and estrogen. Cholesterol,

growth hormone, and insulin levels are often used as indicators of adequate metabolic function. Cholesterol is a precursor to steroid hormone synthesis. Increased levels of circulating cholesterol have been hypothesized to be the cause of higher P_4 levels. Growth hormone and insulin have been associated with follicular recruitment and development (Fortune et al., 2003). Dominant follicle development is largely under the control of the positive and negative feedback of E_2 and stimulation of LH. Two authors have reported conflicting data regarding the effects of fat supplementation on ovarian synthesis of estrogens in beef cattle (Hightshoe et al., 1991; Lammoglia et al., 1997). Hightshoe et al (1991) reported lowered concentration of E-17β in multiparous Simmental cows in an induced first postpartum estrous cycle in response to dietary supplementation with Megalac; while, Lammoglia and co-workers (1997), utilizing Brahman cows in the second postpartum estrous cycle, reported greater E_2 concentrations in response to supplementation with rice bran. The results of Hightshoe et al (1991) are in accordance with those of Echternkamp and Hansel (1973) who reported low E-17β concentrations during the first postpartum estrous in lactating, Holstein cows. Kyle et al (1992) suggested that endocrine balances required for normal reproductive function are restored gradually after parturition. Thus, the results reported by Hightshoe et al (1991) may not be at odds with those of Lammoglia et al (1997). However, data reported on these factors is inconclusive.

The effects of fat supplementation in cattle are well documented. However, studies reporting reproductive responses to long chain, polyunsaturated fatty acid supplementation are inconclusive (Table 2.1) and warrant further examination.

Table 2.1. Effects of fatty acid supplementation on reproductive responses

Reference	Total n	Treatment	Results
Thatcher et al., 2001	--	BEND cells cultured with EPA, DHA, or linolenic acid	EPA, DHA inhibited $PGF_{2\alpha}$ release*
Thatcher et al., 1997	15	Dairy cow diets supplemented with FM vs. no supplement	FM inhibited PGFM*
Mattos et al., 2002	32	Dairy cow diets supplemented with 0, 2.6, 5.2, or 7.8% FM	FM inhibited PGFM* and decreased regression time* No effect on P_4
Mattos et al., 2004	26	Dairy cow diets supplemented with FO or olive oil	FO decreased PGFM* and glucose*
Wamsley et al., 2005	25	Angus heifer diets supplemented with FM or corn gluten control	FM decreased PGFM* in heifers with low P_4 No differences in P_4
Petit et al., 2002	4	Dairy cow diets supplemented with FO:linseed, Meg, formaldehyde treated whole linseed, or duodenally infused linseed oil (latin square)	FO:linseed increased PGFM† No difference in cholesterol Plasma glucose lower for Meg supplemented cows FO:linseed increased class 2 follicles† and size of largest follicle†
Burke et al., 1997	641	Dairy cow diets supplemented with FM vs. no supplement	FM decreased proportion of cows responsive to $PGF_{2\alpha}$* and increased P_4* FM increased pregnancy rate†
Lammoglia et al., 1997	19	Brahman cow diets supplemented with rice bran vs. no supplement	Rice bran increased P_4*, E_2*, and insulin* No difference in cholesterol, PGFM, or growth hormone overall Rice bran increased the number of medium follicles†
Hightshoe et al., 1991	12	Simmental cow diets supplemented with Meg vs. no supplement	Meg increased P_4, LH†, and cholesterol*, follicular growth* Meg lowered E-17β*
Garcia-Bojalil et al., 1998	45	Dairy cow diets supplemented with Meg and degradable intake protein (2x2 factorial)	Meg increased number of CL†, size of the largest CL†, and total luteal area†
Bottger et al., 2002	36	AngusXGelbvieh cow diets supplemented with either high linoleate or oleate safflower seeds vs. no supplement	Treatment did not effect insulin, growth hormone, or glucose
Konrad et al., 1996	--	B-TC3 cells incubated with EPA or no EPA	EPA increased insulin secretion* but did not effect glucose metabolism
Thomas et al., 1997	27	BrahmanXHereford cow diets supplemented with animal tallow (SAT), soybean oil (PU), or fish oil (FO) vs. no supplement	Treatment increased cholesterol* and growth hormone* over time PU*, SAT† and FO† increased number of medium size follicles PU increased insulin over other groups*
Defrain et al., 2005	40	Dairy cow diets supplemented with CS and PP, Meg and PP, or high PP and Meg vs. control	Treatment did not effect glucose or insulin

† ($0.05 < P \leq 0.10$)

* ($P \leq 0.05$)

Abbreviations: (BEND) bovine endometrial cells; (EPA) eicosapentanoic acid; (DHA) docosahexanoic acid; (FM) fish meal; (PGFM) 13,14-dihydro-15-keto-$PGF_{2\alpha}$; (FO) fish oil; (P_4) progesterone; (Meg) Megalac®; (E_2) estrogen; (E-17β) estradiol-17β; (CL) corpus luteum; (β-TC3) β-TC3 pancreatic insulinoma cells; (SAT) saturated fatty acid; (PU) polyunsaturated fatty acid; (CS) corn starch; (PP) propionate pellets

Chapter 3: Rationale and Experimental Objectives

Early embryonic mortality accounts for 75 to 80% of all embryonic and fetal deaths in beef cattle and approaches 30% between days 8 and 16 in beef heifers (Diskin and Sreenan, 1980; Sreenan and Diskin, 1983). Increased embryonic mortality results in more services per conception in dairy cattle; and, more open cows in beef herds due to the restricted length of the breeding season.

Metabolic status has a significant impact on reproductive efficiency in cattle. Of particular concern are developing heifers and postpartum cows which have higher energy requirements than cattle at other stages of development. Inadequate nutrition results in metabolism of bodily stores and tissues both for maintenance and production purposes. While body condition score is one of the most important factors influencing length of the postpartum interval to estrus, nutritional restriction or challenge has also been associated with a decrease in dominant follicle size, delayed ovulation, increased failure to ovulate, and delayed resumption of normal luteal activity (Randel, 1990; Lalman et al., 1997; Mackey et al., 1999; de Vries and Veerkamp, 2000). Conversely, increased energy and dry matter intake has been shown to increase follicular recruitment and dominant follicle size, shorten periods of postpartum anestrous, and increase pregnancy rates (Randel, 1990; Gutierrez et al., 1997; Ciccioli et al., 2003).

Supplementary fats are added to cow diets to increase dietary energy density and improve reproductive efficiency (Drackley et al., 2003; Funston, 2004). The sources of fat supplementation trials in beef cattle are varied. Funston (2004) summarized several studies, detailing effects of various sources of fat supplementation to beef cattle on metabolic hormone secretion, cholesterol and P_4 concentrations, follicular development, $PGF_{2\alpha}$ release, estrus response, and pregnancy rates. Results among studies were highly varied. However, some studies have indicated that positive increases in the above reproductive factors may be due to the fatty acid composition of the diets

rather than simply energy supplementation (Lucy et al., 1991a; Mattos et al., 2002; Petit et al., 2002).

Polyunsaturated fatty acids such as linolenic acid, DHA, and EPA appear to reduce early embryonic mortality by reducing production of $PGF_{2\alpha}$ and enhancing P_4 production; thereby, creating a more favorable uterine environment for the embryo. Recently, inclusion of supplements containing DHA and EPA to diets of lactating dairy cows decreased embryonic death and enhanced pregnancy rates (Staples et al., 1997; Garcia-Bojalil et al., 1998). Little research has been conducted to determine effects on conception or pregnancy rates in beef heifers or cows, the estrous characteristics of beef heifers being similar to those of early post partum beef cows.

Dietary supplementation of EPA and DHA has been reported to increase follicular diameters and circulating E_2 (Hightshoe et al., 1991; Lammoglia et al., 1997). Because E_2 feedback controls development of the dominant follicle and phenotypic estrus response is controlled by E_2, therefore estrus response may be an indirect indicator of follicular development and function.

The objective of this research was to quantify the effects of dietary supplementation of rumen protected Menhaden FM with high levels of EPA and DHA on reproductive factors in beef cattle. The goals of Experiment 1 were to examine the effect of EPA and DHA supplementation on follicular development, luteal phase P_4 concentrations, and circulating levels of PGFM. The goals of Experiment 2 were to determine the effect of EPA and DHA supplementation on concentrations of circulating fatty acids and to determine estrus response, conception rates, and pregnancy rates.

The hypothesis of Experiment 1 was that supplementation of EPA and DHA not alter follicular recruitment and development, serum concentrations of P_4, and release of $PGF_{2\alpha}$ after an OT challenge. The hypothesis of Experiment 2 was that supplementation with EPA and DHA would not increase circulating levels of EPA and DHA or affect estrus response, conception rates, or pregnancy rates in heifers.

Chapter 4: Materials and Methods

Experiment 1

Experiment 1 was conducted at the Beef Reproduction Center at the VPI & SU Kentland Farm in Blacksburg, Virginia (37° 12′ 1.7″ N, 80° 33′ 49.4″ W). Thirty-one non-lactating Angus and Angus-cross cows were used. All cows had produced calves previously. This group of cows had not been rebred so as to provide a pool of non-gravid cows for reproductive research. Three replicates were created. Cows were randomly assigned to dietary treatment and to replicate within treatment. Treatment was either concentrate with fish meal (FM) or concentrate without fish meal (C). Mean initial age, BCS, and BW of cows in the C group were 6.6, 6.8, and 676.6 kg, respectively. Mean initial age, BW, and BCS of cows in the FM group were 6.5, 6.6, 678.2 kg, respectively.

The day supplementation was initiated, within a replicate, was designated experimental day 0 (d 0). Cows were allowed a 2 wk acclimation period prior to d 0 during which they were sorted, penned, and fed individually each day. To facilitate cannulation and blood collection on the final day of the experiment, Rep 2 and Rep 3 were initiated 8 and 12 d after initiation of Rep1, respectively.

Cows were maintained on mixed fescue and clover pasture with ad libitum access to water and mineral mix (appendix III). Animals were confined to a dry lot each evening by 2000 until they were sorted, penned, and individually fed the following morning beginning at 0700. Orts were removed upon sustained refusal and weights recorded. Beginning on d 0, animals were weighed every 2 wk following the morning feeding. Cows were returned to the pasture each day after all animals had received supplemental feed.

Experimental concentrates were designed to be iso-caloric and iso-nitrogenous (Table 4.1). Rumen protected FM was supplemented in the form of EnerGII Reproduction Formula (Virtus

Nutrition LLC, Fairlawn, Ohio) and was provided at 227.3 g^{-1}·cow $^{-1}$·day for the first week. EnerGII was then increased to and held at 454.5 g $^{-1}$·cow $^{-1}$·day until the conclusion of the experiment.

Table 4.1. Calculated composition of C and FM supplements fed daily (Experiment 1)[1]

	IFN[2]	C (kg/cow)	FM (kg/cow)
Cracked Corn	4-20-698	1.59	1.14
Soybean Meal	5-04-612	0.00	0.08
Molasses	4-04-696	0.23	0.23
Limestone	6-02-632	0.13	0.00
EnerGII[3]		0.00	0.46
Total	kg as fed	1.95	1.90
	NE_m[4]	3.62	3.66
	NE_g[4]	2.64	2.68
	CP (kg)	0.15	0.15

[1] Cows in treatment (FM) groups received a rumen protected fish meal supplement. Cows in control (Control) groups did not receive the supplement.
[2] International feed number.
[3] EnerGII Reproduction Formula (Virtus Nutrition LLC, Fairlawn, OH)
[4] Net energy for maintenance (NE_m); net energy for gain (NE_g); Mcal

Concentrates and pasture were sampled weekly and shipped to Cumberland Valley Analytical Services, Inc. in Hagerstown, Maryland to be analyzed for CP, ADF, and crude fat. Values of net energy for maintenance (NE_m) and net energy for gain (NE_g) were calculated using the following equations: $NE_m = 1.37 * ME – 0.138 * ME^2 + 0.0105 * ME^3 -1.12$; $NE_g = 1.42 * ME – 0.174 * ME^2 + 0.0122 * ME^3 – 1.65$ (NRC, 1996). Batch samples were collected each time C or

FM concentrates were formulated; however, no analysis was conducted due to misplacement of the samples. Unless otherwise noted, dietary data presented is as analyzed.

Cows were estrous synchronized using the Ovsynch protocol with CIDR (Pursely et al., 1995). On d 28 cows were injected with 100 μg of GnRH (Fertagyl®, Intervet Inc., Millsboro, DE) and a CIDR (1.38 g P_4 Pfizer Inc., New York, NY) was inserted. On d 35 CIDRs were removed, and cows were injected with 25 mg of $PGF_{2\alpha}$ (Lutalyse, Pfizer Inc., New York, NY), followed by 100 μg of GnRH to induce ovulation on d 37.

Ovaries of all cows were examined by trans-rectal, B-mode, real time ultrasound (Aloka SSD500V, Corometrics Medical Systems, Inc., Wallingford, CT) with a 5-MHz linear array transducer on days 35, 37 and 40. Ovaries were examined on d 35 and d 37 to determine the diameter of the largest follicle and evaluate follicular growth from d 35 (2 d prior to ovulation) to d 37 (day of ovulation). Follicular growth was defined as the change in follicular diameter from d 35 to d 37. Ovaries were examined on d 40 to confirm ovulation. All ultrasound examinations were recorded using a VHS recorder. Data was then digitalized and converted to digital video disc format using Ulead Video Studio 8.0 (Ulead Systems, Taiwan) and Nero 7 (Nero Inc., Glendale, CA). Files were viewed using Cyberlink PowerDVD (Cyberlink USA, Freemont, CA) and screenshots selected. The area of the largest follicle was determined using ImagePro Plus 4.5 (Image Processing Solutions Inc., North Reading, MA) and maximum diameters were calculated from the area of the follicle. Five animals were dropped from the data analysis for follicular growth and size because of incomplete individual records due to malfunctioning VHS equipment.

Blood samples (10 mL) were collected on d 17 and d 28 and serum P_4 concentrations were determined to verify cyclicity. Cyclicity was defined as having a P_4 concentration greater than 1.0 ng/mL at either d 17 or d 28. From d 37 through d 52 blood samples were collected by jugular venipuncture to determine serum P_4 profiles (corresponding to d 0 to d 15 after ovulation).

Following collection, blood samples were allowed to coagulate for 1 h at room temperature. Blood samples were subsequently centrifuged at room temperature for 15 min at 1200 x g (Clay Adams Dynac Centrifuge and Clay Adams Safeguard Centrifuge, Becton-Dickenson Co., Parsippany, NJ). Serum was decanted and stored at -20 °C.

Intensive blood collection was performed on d 52 (15 d after induced ovulation) to determine effects of supplementation on $PGF_{2\alpha}$. Cows were estrogen primed beginning at 0430 with a 6 mL intravenous injection containing 0.5 mg/mL estradiol-17β [saline:ethanol (50:50)]. Release of $PGF_{2\alpha}$ was stimulated by an intravenous injection of OT (100 IU; Vedco Inc., St. Joseph, MO) at 0830. Jugular catheterization began at 0600. Blood samples (10 mL) were collected at 15-min intervals from 30 min before OT injection to 2 h after injection (-30, -15, 0, 15, 30, 45, 60, 75, 90, 105, and 120 min). Additional samples were collected at 150, 180, and 240 minutes following OT injection. Following collection, blood samples were processed for serum collection and stored as previously described.

Serum P_4 concentrations were quantified by solid-phase radioimmunoassay (Diagnostics Products Corp., Los Angeles, CA). Sensitivity of the assay was 0.02 ng/mL. Inter-assay coefficient of variation was 10.05%. Intra-assay coefficient of variation was 5.54%.

Blood samples were analyzed for PGFM concentration at the United States Department of Agriculture, Agricultural Research Service, Fort Keogh Livestock and Range Research Laboratory in Miles City, MT using procedures (Appendix IV) adapted from Silvia and Niswender (1984) and Homanics and Silvia (1988). Blood samples were packed in dry ice and shipped via next day air from Blacksburg, VA to Miles City, MT where they were placed in storage at -20 °C until assayed. The sensitivity of the assay was 11.50 pg/mL. Intra- and inter-assay coefficients of variation were 6.42% and 3.79%, respectively.

Statistics. Reproductive responses were characterized with respect to daily and overall intake. No interaction was found between intake and reproductive response.

Ovarian follicular size and growth were analyzed by analysis of variance using the GLM procedure of SAS (Statistical Analysis System v9.1, Cary, NC). The use of general linear models assumes that each dependent variable is independent of other dependent variables and is the product of a linear function of the independent variables and an error component with constant variability. Treatment and replication were included as main effects. The interaction was removed because it did not reach a significance level of $P = 0.10$.

Progesterone concentrations were analyzed by analysis of variance for repeated measures using the MIXED procedures of SAS (Statistical Analysis System v9.1, Cary, NC). The MIXED procedures generalize standard linear models, allowing dependent variables to exhibit correlations with other dependent variables and assuming non-constant variability of the error component due to unknown random effects. Treatment, replication, and day were included as main effects. All main effects and interactions were included in the model. Backwards selection for model fit was not performed because of the presence of a significant three-way interaction. Covariance structures were compared for goodness of fit using the Schwarz Bayesian criterion. Subsequently, variances and correlations were modeled as unstructured. Individual progesterone concentrations for each day were analyzed using the GLM procedures of SAS. Treatment and replication were included as main effects. Interactions were removed because they failed to meet the significance level of $P = 0.10$.

Concentrations of PGFM were analyzed by analysis of variance for repeated measures using the MIXED procedures of SAS. Treatment, replication, and time were included as main effects as well as all interactions. The model was reduced by backwards selection until only the main effects were present in the model. The significance level was set at $P = 0.10$ for removal from the model.

Covariance structures were compared for goodness of fit using the Schwarz Bayesian criterion. Subsequently, variances and correlations were modeled as autoregressive. Individual times were analyzed by analysis of variance using the GLM procedures of SAS as a multivariate linear model with treatment and replication as the main effects. Tukey's multiple comparison test was used to compare main effect means.

Area under the PGFM curve was approximated using the trapezoidal rule to serve as a measure of total PGFM output (Galyean, 1993). Area under the curve was analyzed using the GLM procedures of SAS. Treatment and replication were included in the model. Basal concentrations of PGFM, peak concentrations of PGFM, and the difference from basal to peak levels were analyzed using the GLM procedures of SAS. Time, replication, and time x replication were included in the model. Basal concentration was defined as the mean PGFM concentration from -30 min to 0 min relative to oxytocin injection.

Five animals were characterized as not having responded to the oxytocin injection within 45 min post injection. Response to oxytocin was defined as a PGFM concentration occurring within 45 min post oxytocin administration that was greater than or equal to twice the mean basal concentration of PGFM. Animals that did not respond were removed from the data set prior to statistical analysis. These data are presented separately.

Experiment 2

Experiment 2 was conducted at the Virginia Department of Corrections Southampton Correctional Facility near Capron, Virginia (36° 43′ 20.2″ N, 77° 15′ 19.6″ W). Angus crossbred heifers were weighed. Heifers were reproductive tract scored via rectal palpation and pelvic areas measured with a caliper type Rice pelvimeter (Lane Manufacturing, Denver, CO; Johnson et al., 1988; Anderson et al., 1991). Heifers with BW < 227.3 kg at the time of evaluation, a reproductive

tract score of 1, or a pelvic area less than 150 cm^2 were removed from the experiment. The remaining heifers (n = 214) were blocked by weight into light or heavy groups (light $\leq$ 295.5 kg; heavy > 295.5 kg) and randomly assigned within weight group to dietary treatment. Dietary treatments consisted of corn silage based diets supplemented with rumen protected Menhaden fish meal (FM) in the form of EnerGII Reproduction formula or concentrate without fish meal (C). Thus, experimental groups consisted of the following: heavy weight-FM (n = 50), heavy weight-C (n = 51), light weight-FM (n = 55), and light weight-C (n = 58).

Heifers assigned to each diet were group fed a total mixed ration containing the concentrate (3.6 $kg \cdot hd^{-1} \cdot d^{-1}$) and corn silage (11.4 $kg \cdot hd^{-1} \cdot d^{-1}$) and were offered hay *ad libitum*. The nutrient content of both the concentrates and concentrates - corn silage mixes are shown in Tables 4.2 and 4.3. Experimental groups were fed and penned separately. Diets were fed for 54 d, beginning 30 d before initiation of estrus synchronization and ending 14 days after last day of insemination. Day of diet initiation was designated experimental day 0 (d 0). Figure 4.1 indicates the complete experimental calendar for Experiment 2.

Both the concentrates and total diets were sampled and stored at -20°C prior to shipping to Cumberland Valley Analytical Services, Inc. in Hagerstown, Maryland to be analyzed for CP, ADF, and crude fat. Values of net energy for maintenance (NE_m) and net energy for gain (NE_g) were calculated using the following equations: $NE_m = 1.37 * ME - 0.138 * ME^2 + 0.0105 * ME^3 - 1.12$; $NE_g = 1.42 * ME - 0.174 * ME^2 + 0.0122 * ME^3 - 1.65$ (NRC, 1996). The presented nutrient composition of diets is as analyzed.

Table 4.2. Analyzed nutrient composition of concentrates and total mixed rations (Experiment 2)

	DM	CP	CF[1]	ADF	NE_m[1]	NE_g[1]
Concentrates[2]	%	%	%	%	Mcal/kg	Mcal/kg
C[2]	85.2	15.4	3.5	4.8	0.87	0.58
FM[2]	82.7	13.2	5.5	4.3	0.87	0.58
Total Mixed Ration[3]						
C[2]	44.1	12.8	3.3	16.6	0.83	0.54
FM[2]	35.5	8.6	4.4	20.6	0.81	0.53

[1] Crude fat (CF); net energy for maintenance (NE_m); net energy for gain (NE_g)
[2] The concentrate grain mix consisted of corn and soybean meal with (FM) or without (C) EnerGII Reproduction formula (Virtus Nutrition LLC, Fairlawn, Ohio)
[3] Concentrate grain mix and corn silage

Table 4.3 Analyzed dietary nutrient composition offered to each heifer daily (Experiment 2)

	DM	CP	CF[1]	ADF	NE_m[1]	NE_g[1]
Concentrate[2]	(kg)	(kg)	(kg)	(kg)	(Mcal)	(Mcal)
C[2]	3.1	0.48	0.11	0.15	2.7	1.8
FM[2]	3.0	0.41	0.17	0.13	2.6	1.8
Total Mixed Ration[3]						
C[2]	5.0	0.64	0.16	0.83	4.2	2.7
FM[2]	4.0	0.43	0.22	1.0	3.3	2.1

[1] Crude fat (CF); net energy for maintenance (NE_m); net energy for gain (NE_g)
[2] The concentrate grain mix consisted of corn and soybean meal with (FM) or without (C) EnerGII Reproduction formula (Virtus Nutrition LLC, Fairlawn, Ohio)
[3] Concentrate grain mix and corn silage (CS)

Estrus was synchronized using the modified CIDR-PG protocol shown in Figure 4.1 (Hall, 2005). Estrus detection was performed using the HeatWatch electronic estrus detection system (CowChips, LLC., Denver, CO). On d 30 a CIDR (1.38 g P_4 Pfizer Inc., New York, NY) was inserted intravaginally in each heifer. Six days later (d 36) $PGF_{2\alpha}$ (25 mg; Lutalyse, Pfizer Inc., New York, NY) was administered to each heifer via intramuscular injection and HeatWatch transmitters were attached. The following day (d 37) CIDRs were removed. Estrus response was defined as the occurrence of estrus within 72 h after CIDR removal. Estrus was defined as a minimum of three mounts with a minimum duration of two seconds detected within a 4-h period with maximum mount duration of 30 sec and heat exclusion duration of 48 h. Heifers detected in estrus were artificially inseminated with semen from one of two Angus bulls approximately 12 h after initiation of estrus. In addition, semen from one bull (Bull A) was tested with 2 different extenders which were identified only by semen straw color (green or orange). The contents of the semen extenders used are unknown. Semen from the AI sire involved in the semen extender study was distributed evenly over the four groups and randomly among the heifers. Due to a supply error, the AI sire (Bull B) which was not part of the semen extender study was not utilized for heifers in the L-FM group. Two technicians inseminated all the heifers. Technician was distributed evenly over the four groups and randomly among the heifers. Heifers not exhibiting estrus by 72 h post CIDR removal were administered 100 μg of GnRH (Cystorelin, Merial Ltd., Duluth, GA) and artificially inseminated at that time by fixed-time AI (TAI). Two weeks post-AI, natural service sires were placed with heifers to breed heifers not pregnant to AI. Each natural service sire was allocated 40 to 50 heifers. Heifers were randomly placed with natural service sires without regard to previous dietary treatment or weight group. Thus, natural service sire, dietary treatment, and weight group are confounded during the natural service period.

Pregnancy diagnosis was conducted by trans-rectal ultrasonography at 60 d post-AI and confirmed by rectal palpation 90 d post AI. Conception rate was defined as the number of heifers that became pregnant divided by the number of heifers presented to be bred. Conception rates for AI were differentiated between those heifers bred after an observed estrus versus those heifers which were not observed in estrus and were administered GnRH and bred by TAI. Pregnancy rate was defined as the number of heifers that became pregnant divided by the total number of heifers in the study. All heifers in the study were presented for AI, either after an observed estrus or by TAI. Therefore, total AI pregnancy rate was defined as the total number of heifers pregnant to AI divided by the number of heifers in the study. Overall pregnancy rate was defined as the number of heifers that became pregnant, whether to AI or natural service, divided by the number of heifers in the study.

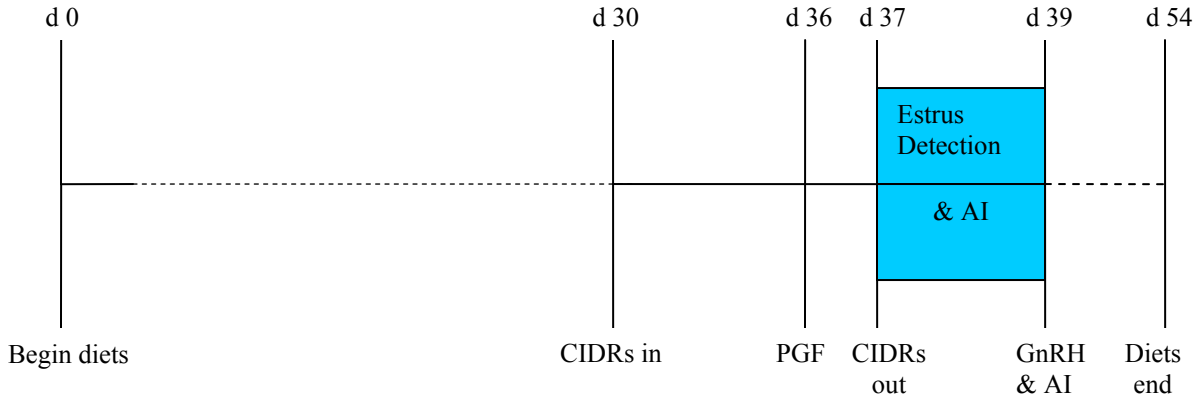

Figure 4.1. Diagrammatic representation of experimental design in Experiment 2. Day of diet initiation was designated experimental day 0 (d 0). Dietary treatments consisted of corn silage based diets supplemented with rumen protected Menhaden fish meal in the form of EnerGII Reproduction formula or concentrate without fish meal. Diets were fed for 54 d, beginning 30 d before initiation of estrous synchronization. Diets were continued 14 d post-AI. Estrous cycles were synchronized using a modified CIDR-PG protocol. Heat detection and AI were conducted for 72 h post CIDR removal. Heifers not observed in estrus were injected with GnRH and bred. Clean up bulls were placed with the heifers post-AI.

In each group, blood samples (10 mL) for analysis of fatty acid content were collected on d 36 from five randomly selected. Samples were collected in silicate anti-coagulation vacutainer tubes (Vacutainer, Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) and placed on ice. Blood samples were transported on ice for 4.5 h to the laboratory and centrifuged at 4 °C for 20 min at 1800 x g (Sorvall RC-3B Refrigerated Centrifuge, Thermo Electron Corp., Asheville, NC). Plasma aliquots were decanted and stored at -20 °C pending analysis. Plasma samples were analyzed for fatty acid composition by the Dairy Nutrition Laboratory in the Department of Dairy Science at Virginia Polytechnic Institute and State University using the following procedures.

Extraction and Methylation. Fatty acids were extracted using modified Folch procedure (Folch et al., 1957). One mL aliquots of serum were extracted with 2:1 (v/v) chloroform:methanol. Samples were allowed to stand for a minimum of 2 h and filtered through Whatman 1 filter paper. Residue was washed twice with 2:1 chloroform:methanol. Crude lipid extract was washed with 0.88 % KCl for 10 min on a horizontal platform shaker at medium/high speed. Samples were centrifuged (IEC GP8R) at 930 x g for 5 min. The aqueous layer was aspirated and solvent volume reduced to 2 mL under a stream of nitrogen in an N-Evap (Organomation, Inc) at 40 °C. Residue was transferred to 16 x 100 mm glass methylation tubes. Extraction tube was washed twice with 2:1 chloroform:methanol and added to methylation tube. Methylation tube volume was reduced to dryness under nitrogen. Fatty acids were trans-esterified to methyl esters with 0.5 N NaOH in methanol and 14 % BF_3 (Park and Goins, 1994). Ten-heptadecenoic acid (Nu-Check Prep) was added as an internal standard.

Chromatographic analysis. All samples were analyzed on a 6890N gas chromatograph with a 7683 autoinjector, split/splitless capillary inlet and flame ionization detector (Agilent Technologies, Palo Alto, CA). Carrier gas was ultrapure H_2, gas velocity 29 cm/sec, flow 1.6 mL/min, injection volume 0.5 µL, splitless injection with 0.6 min purge valve closure. A

Chrompack CP-Sil 88 100 m x .25mm id capillary column was used to separate FAME (Varian Associates, Palo Alto, CA). Temperature program for separations began at 40 °C, held for 0.6 min, increased to 100 °C at 40 °C/min, held for 10 min, increased to 175 °C at 25 °C/min, held for 45 min, increased to 220 °C at 10 °C/min and held for 19 min. Total runtime was 83.6 min. Temperatures for injector and detector were 250 °C and 300 °C, respectively. Data were integrated and quantified using a Chem DataStation (Agilent Technologies, Palo Alto, CA).

Statistics. Concentrations of arachadonic acid, EPA, and DHA were analyzed by analysis of variance using the GLM procedures of SAS. Tukey's multiple comparison test was used to compare main effect means. Treatment and weight group were included in the model. The interaction was removed because it did not reach a significance level of $P = 0.10$.

The interval from CIDR removal to estrus in heifers that exhibited estrus after estrus synchronization was analyzed by analysis of variance using the GLM procedures of SAS. Treatment and weight group were included as main effects. The interaction was not tested because insufficient degrees of freedom existed within the model.

Estrus response was analyzed by analysis of variance using the GLM procedures of SAS. Treatment and weight group were included as main effects. The interaction was not tested because of insufficient degrees of freedom.

Conception and pregnancy rates as affected by dietary treatment and weight group were analyzed by analysis of variance using the GLM procedures of SAS. Treatment and weight group were included as main effects. The interaction was not tested because of insufficient degrees of freedom. Conception and pregnancy rates as affected by technician and AI sire were analyzed using the LOGISTIC procedures of SAS (Statistical Analysis System v9.1, Cary, NC) for binary data. Linear regression, such as used for general linear models, predicts the value of the dependent variable as a linear function of the independent variable(s). Logistic regression seeks to predict the

probability of the subject doing or being, while assuming that the probability as the dependent variable is linearly related to a function of the independent variable(s). Heifers bred to one AI sire were removed from the data set because that sire was not present in all weight groups. Technician and AI sire were included in the model as main effects as well as the interaction. The model was reduced by backwards selection until only the main effects were present in the model. The significance level was set at $P = 0.10$ for removal from the model.

Chapter 5: Results

Experiment 1

Cyclicity. All cows were cyclic prior to the beginning of estrus synchronization treatment during the experiment as indicated by a serum P_4 concentration greater than 1 ng/mL at experimental d 17 or d 28.

Supplement Intake. Intake of the concentrate varied among cows in Experiment 1 (Table 5.1). Cows receiving the FM or C concentrates were offered 1.90 $kg \cdot hd^{-1} \cdot d^{-1}$ and 1.95 $kg \cdot hd^{-1} \cdot d^{-1}$, respectively. Mean nutrient compositions of the orts are given in table 5.2.

Table 5.1. Intake of concentrate by dietary treatment (Experiment 1)[1]

	Mean Intake ($kg \cdot hd^{-1} \cdot d^{-1}$)	Minimum Total Intake[2] ($kg \cdot cow^{-1}$)	Maximum Total Intake[2] ($kg \cdot cow^{-1}$)
C	1.89 ± 0.01	120.6	123.5
FM	1.87 ± 0.1	98.5	126.8

[1] Cows in treatment (FM) groups received a rumen protected fish meal supplement. Cows in control (C) groups did not receive the supplement.
[2] Total intakes (sum of daily intakes) for the entire experimental period (d 0 to d 52)

Table 5.2 Mean analyzed nutrient composition of concentrate orts by dietary treatment (Experiment 1)

	DM	CP	Fat	ADF	NE_m	NE_g
	%	%	%	%	Mcal/kg	Mcal/kg
C[1]	83.6	9.9	4.9	4.3	1.1	0.74
FM[1]	85.8	8.9	10.3	4.1	1.1	0.75

[1] Cows in treatment (FM) groups received a rumen protected fish meal supplement. Cows in control (C) groups did not receive the supplement.

Ovarian Dynamics. There was no effect of treatment or replication on ovarian dynamics (Table 5.3). Dietary treatment did not affect the size of the largest follicle on d 35 (P = 0.59) or d 37 (P = 0.36). Also there was no difference in growth in diameter of the pre-ovulatory follicle from d 35 to d 37 (P = 0.72).

Table 5.3. Effect of FM or C supplement on mean (± SE) follicular diameter and growth (Experiment 1)[1]

	C Diameter (mm)	FM Diameter (mm)	*P*
LF 35[2]	15.2 ± 1.1	13.8 ± 1.3	0.59
LF 37[3]	16.5 ± 1.0	15.0 ± 1.1	0.36
GOF[4]	3.2 ± 1.0	1.7 ± 1.1	0.72

[1] Cows in treatment (FM) groups received a rumen protected fish meal in a concentrate supplement. Cows in control (C) groups received a concentrate without fish meal.
[2] Size of the largest follicle on d35.
[3] Size of the largest follicle on d 37 regardless of ovary.
[4] Growth from d35 to d37 of the pre-ovulatory follicle.

Progesterone. Mean P_4 concentrations from d 2 to d 16 of the synchronized estrous cycle are shown in Figure 5.1. A three-way interaction was found between the effects of treatment, replication, and day using the MIXED procedures of SAS. The interaction was explored by evaluating differences in P_4 concentrations due to dietary treatment or replication on individual days. The effect of replication tended to be significant on d 4 (P = 0.07) and was significant on d 5 (P = 0.02). On d 4 and d 5 mean P_4 concentrations were greater in serum collected from animals in the first replicate than for those in replicates 2 and 3 (0.49 ± 0.70 ng/mL vs. 0.09 ± 0.23 ng/mL and

0.17 ± 0.52 ng/mL; 0.91 ± 0.52 ng/mL vs. 0.53 ± 0.35 ng/mL and 0.44 ± 0.44 ng/mL, respectively). Within replicate 1 on d 4 and d 5 mean P_4 concentrations were greater for cows receiving rumen protected fish meal than for cows receiving the C concentrate (0.69 ± 0.78 ng/mL vs. 0.29 ± 0.34 ng/mL; 0.97 ± 0.68 ng/mL vs. 0.84 ± 0.26 ng/mL, respectively).

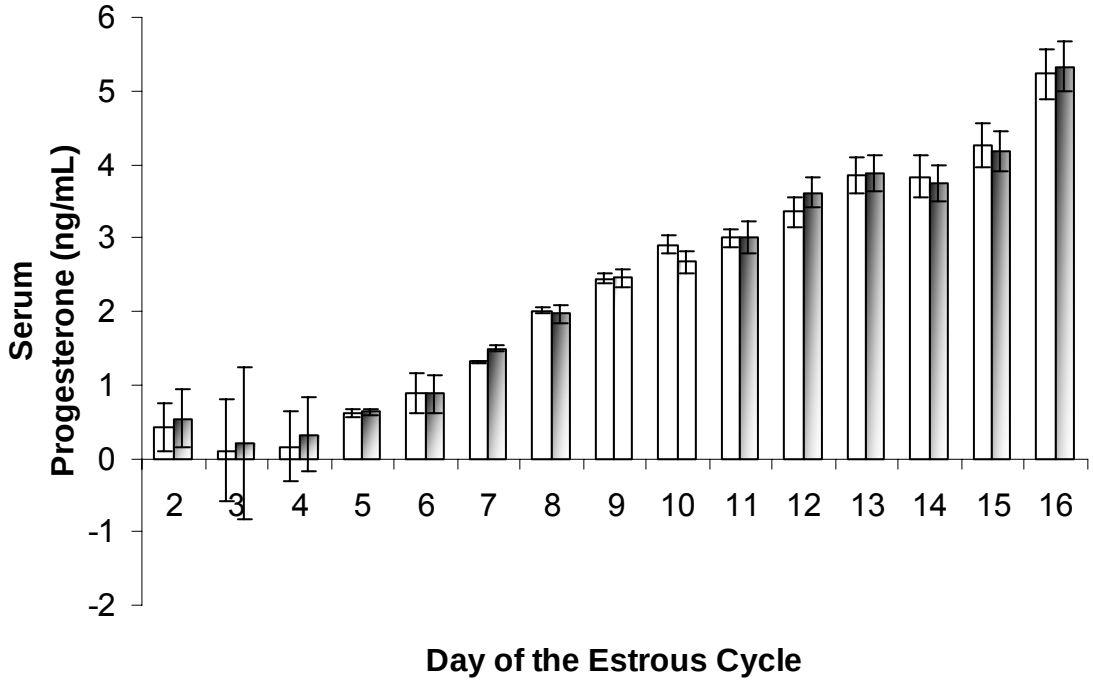

Figure 5.1. Mean progesterone concentrations from d 2 (d 38) of the synchronized estrous cycle through d 16 (d 52) for cows fed rumen protected fish meal (■) and control cows (□). No effect of supplement (P > 0.10); effect of time (P < 0.05).

PGFM. Mean concentrations of PGFM for cows receiving rumen protected FM were numerically lower (P = 0.13) than for those cows not receiving FM for all times except 120 and 240 min after oxytocin injection (Figure 5.2). Mean overall PGFM concentrations were greater (P = 0.03) for cows in the first replicate than for those in replicates 2 and 3 at all times except for 90 and

180 min after oxytocin. Mean PGFM levels for time periods with $P \leq 0.16$ are given in tables 5.4 and 5.5.

Table 5.4. Mean (± SE) concentrations of PGFM for FM and C concentrate supplemented cows from 0 min to 60 min after oxytocin injection on d 16 of the estrous cycle[1]

Time[2]	FM[1]	C[1]	*P*
0	112.3 ± 5.6	148.8 ± 5.6	0.06
30	96.2 ± 5.8	110.3 ± 6.3	0.04
45	68.1 ± 4.3	99.1 ± 5.6	0.16
60	75.7 ± 3.8	94.0 ± 4.9	0.05

[1] Cows in treatment (FM) groups received a rumen protected fish meal in a concentrate supplement. Cows in control (C) groups received a concentrate without fish meal.
[2] Minutes after oxytocin injection.

Table 5.5. Comparison of mean (± SE) concentration of PGFM by replicate[1]

Time[2]	Rep1	Rep2	Rep3	*P*
-15	72.1 ± 5.2	29.8 ± 2.6	41.2 ± 2.8	0.02
0	73.5 ± 5.6	34.8 ± 3.0	43.5 ± 3.9	0.04
30	153.8 ± 6.2	110.1 ± 7.7	88.0 ± 4.5	0.06
45	159.8 ± 5.3	62.8 ± 5.0	90.0 ± 4.2	0.003
60	104.5 ± 6.3	53.4 ± 3.6	91.8 ± 4.5	0.07

[1] Replicate 1 (Rep1), replicate 2 (Rep2), replicate 3 (Rep3)
[2] Minutes relative to oxytocin injection.

Total prostaglandin production over time as estimated by area under the PGFM curve did not differ among replicates or between treatments. Total area under the curve tended (P = 0.07) to be less for replicate 2 than for replicates 1 and 3 (98,100.2 units^2 vs. 193,865.3 units^2 and 226,803.6 units^2, respectively). Basal levels of PGFM tended (P = 0.08) to be decreased for cows receiving EnerGII than for C cows (42.8 ± 2.8 pg/mL vs. 56.2 ± 4.5 pg/mL). Mean basal PGFM concentration was greater (P = 0.01) for the first replicate than for the 2nd or 3rd replicates (74.4 ± 4.4 pg/mL vs. 39.0 ± 12.5 pg/mL and 45.4 ± 2.3 pg/mL). Response to oxytocin injection as determined by the difference between basal and peak levels of PGFM did not differ (P=0.56) between treatments (83.9 ± 4.7 pg/mL vs. 108.8 ± 5.4 pg/mL). No difference (P = 0.16) was observed between treatments in peak concentration of PGFM (126.7 ± 4.7 pg/mL vs. 165.0 ± 5.6 pg/mL). Profiles of cows not responding to oxytocin are shown in Figure 5.3 and 5.4.

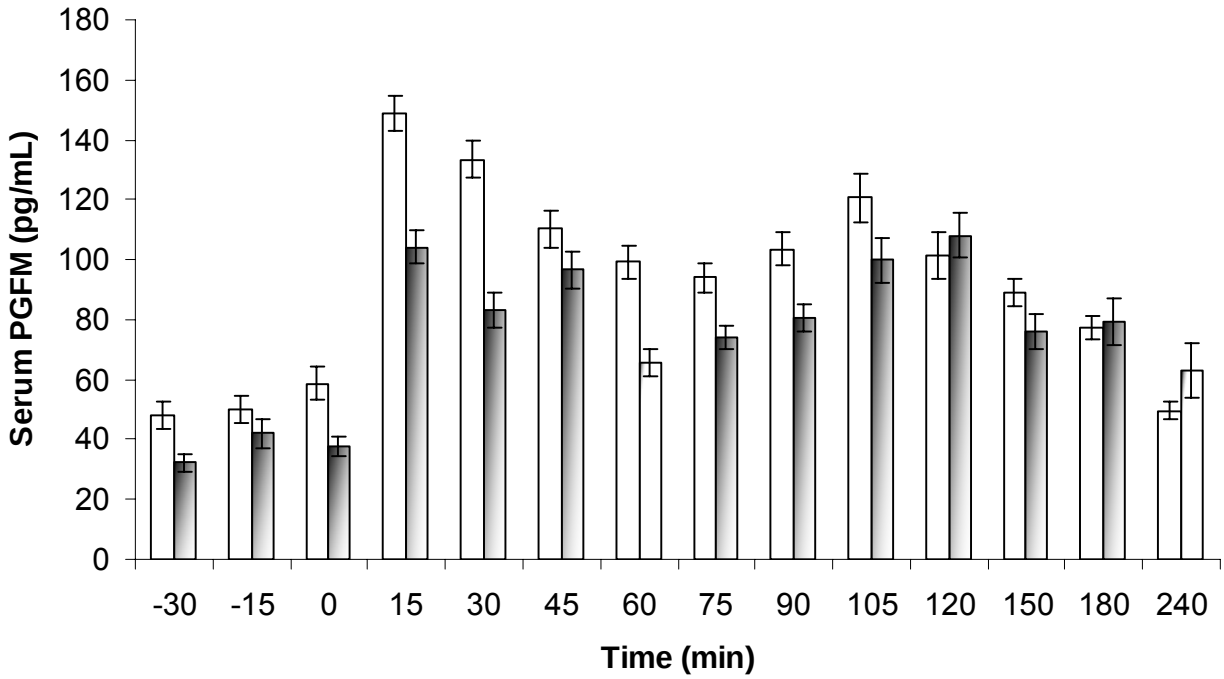

Figure 5.2. Mean PGFM concentrations on d 16 (d 52) of the synchronized estrous cycle for cows that responded to oxytocin injection only (Experiment 1) for rumen protected fish meal (■) and control (□) supplemented cows. Cows were estrogen primed at -240 min. Oxytocin was given at 0 min to elicit prostaglandin release. Effect of supplement (P= 0.13); effect of time (P < 0.05).

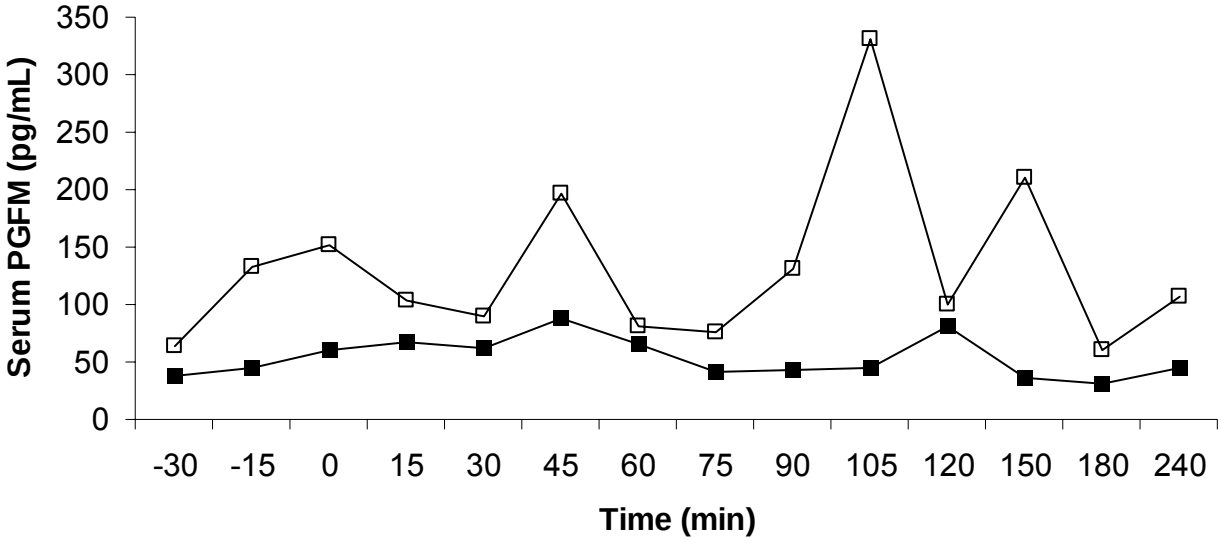

Figure 5.3. Mean PGFM concentrations on d 16 for 2 cows receiving the control supplement and which did not respond to oxytocin injection (Experiment 1). Cows were estrogen primed at -240 min. Oxytocin was given at 0 min to elicit prostaglandin release. Cow 105 is denoted by the line with the open blocks (□). Cow 153 is denoted by the line with the closed blocks (■).

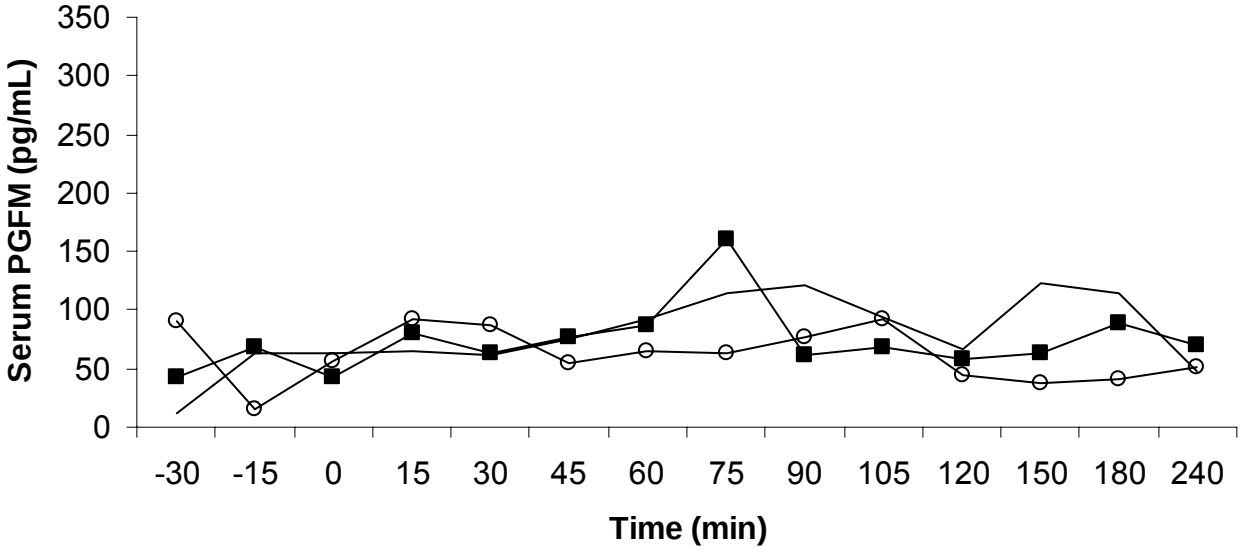

Figure 5.4. Mean PGFM concentrations on d 16 for 3 cows receiving the rumen protected fish meal supplement and which did not respond to oxytocin injection (Experiment 1). Cows were estrogen primed at -240 min. Oxytocin was given at 0 min to elicit prostaglandin release. Cow 162 is denoted by the line with the open circles (○). Cow X64 is denoted with the smooth line (-). Cow X76 is denoted by the line with the closed blocks (■).

Experiment 2

Fatty Acid Profile. Mean plasma concentrations of arachadonic acid, EPA, and DHA are shown in Table 5.6. Supplementation of heifer diets with EnerGII did not affect plasma levels of arachadonic acid ($P = 0.77$). However, concentrations of EPA and DHA were ($P < 0.04$) increased in circulation of animals fed the FM diet when compared to controls.

Table 5.6. Mean (± SE) plasma fatty acid concentration in five heifers receiving and five heifers not receiving rumen protected FM[1]

	C	FM	P
ARCH[2]	18.8 ± 1.1	17.9 ± 2.0	0.77
EPA[2]	3.7 ± 0.8	7.9 ± 1.9	0.04
DHA[2]	1.8 ± 0.5	3.4 ± 0.9	0.02

[1] Heifers in treatment (FM) groups received a rumen protected fish meal in a concentrate supplement. Heifers in control (Control) groups received a concentrate without fish meal.
[2] Arachadonic acid (ARCH), eicosapentanoic acid (EPA), docosahexanoic acid (DHA)

Estrus Response. Of 214 total heifers treated to synchronize estrus, 156 (72.9%) were detected in estrus within 72 hours after CIDR removal. Those heifers were artificially inseminated approximately 12 h after estrus detection. No differences were detected in the interval from CIDR removal to estrus due to supplement feeding (FM 21:59 h vs. C 23:54 h; $P = 0.17$) or due to weight group (L 20:22 h vs. H 18:48 h; $P = 0.33$). Table 5.7 shows overall estrus response by treatment and weight group. Neither dietary treatment nor weight group affected the number of heifers observed in estrus after CIDR removal.

Table 5.7. Percentages of heifers observed in estrus within 72 h after estrus synchronization treatment in Experiment 2

Group	%	P
C[1]	72.5 (79/109)	0.97
FM[1]	73.3 (77/105)	
L[2]	81.4 (92/113)	0.35
H[2]	63.4 (64/101)	

[1] Heifers in treatment (FM) groups received a rumen protected fish meal in a concentrate supplement. Heifers in control (Control) groups received a concentrate without fish meal.

[2] Heifers in the light group (L) had body weights ≤ 295.5 kg. Heifers in the heavy group (H) had body weights > 295.5 kg. Groups were created at the time of reproductive tract scoring and pelvic measurement.

Conception and Pregnancy Rates. Overall pregnancy rate to AI and natural service was 90.2%. No differences due to dietary treatment or weight group were detected for the number of heifers that conceived to artificial insemination after detected estrus or for heifers that conceived to timed AI. Dietary supplementation with FM did not affect overall pregnancy rate to AI (P = 0.95; Table 5.8). The effects of semen extender and AI technician are shown in table 5.9. Semen extender tended (P = 0.11) to affect the conception rate of heifers bred after a detected estrus and total AI pregnancy rate (P = 0.06). Heifers bred with Bull A-Orange were 0.52 times more probable to conceive than those bred with Bull A-Green (95% CI; 0.269 to 1.020). Artificial insemination technician did not affect conception or pregnancy rates (P > 0.05).

Table 5.8. Effect of dietary supplement and weight group on conception and pregnancy rates (Experiment 2)[1]

	Conception Rates						Pregnancy Rates		
	EAI[2]			TAI[3]			Total[4]		
Group	%		P	%		P	%		P
C	53.2	(42/79)	0.67	36.7	(11/30)	0.74	48.6	(53/109)	0.80
FM	55.8	(43/77)		39.3	(11/28)		51.4	(54/105)	
L	53.3	(49/92)	0.60	33.3	(7/21)	0.69	49.6	(56/113)	0.93
H	56.3	(36/64)		40.5	(15/37)		50.5	(51/101)	

[1] Heifers in treatment (FM) groups received a rumen protected fish meal in a concentrate supplement. Heifers in control (C) groups received a concentrate without fish meal. Heifers in the light group (L) had body weights ≤ 295.5 kg. Heifers in the heavy group (H) had body weights > 295.5 kg. Groups were created at the time of reproductive tract scoring and pelvic measurement.
[2] Heifers conceived to artificial insemination of those observed in estrus.
[3] Heifers conceived of those receiving time artificial insemination.
[4] Total heifers pregnant to artificial insemination.

Table 5.9. Effect of semen extender and artificial insemination technician on conception and pregnancy rates (Experiment 2)[1]

	Conception Rates						Pregnancy Rates		
	EAI[2]			TAI[3]			Total[4]		
	%		P	%		P	%		P
Extender									
Bull A-Green	46.3	(42/67)	0.11	33.3	(1/3)	0.35	45.7	(32/70)	0.06
Bull A-Orange	62.7	(31/67)		50.0	(3/6)		61.6	(45/73)	
Technician									
DL	57.4	(39/68)	0.55	40.0	(2/5)	0.97	56.2	(41/73)	0.56
RAM	51.5	(34/66)		50.0	(2/4)		51.4	(36/70)	

[1] Bull A semen was extended with one of two extenders known only by straw color (green or orange). Two AI technicians were used (DL or RAM).
[2] Heifers conceived to artificial insemination of those observed in estrus.
[3] Heifers conceived of those receiving time artificial insemination.
[4] Total heifers pregnant to artificial insemination.

Chapter 6: Discussion

The purpose of this project was to examine the effect of a commercial rumen protected fish meal supplement on ovarian follicular development, progesterone production, and prostaglandin release in beef cows, and concentrations of circulating fatty acids and reproductive performance in nulliparous beef heifers.

Experiment 1

Ovarian Follicular Characteristics. Several authors have reported positive effects of supplementing long chain fatty acids on ovarian follicular numbers and growth in beef and dairy cows. Greater numbers of class II, III, and IV follicles have been reported, as well as, greater numbers of follicles progressing to higher stages, thus avoiding atresia (Lucy et al., 1991a; Hightshoe et al., 1991; Garcia-Bojalil et al., 1998). In addition, supplementation of EPA and DHA, either as fish meal or as products containing their precursors, has been reported to increase the size of the dominant follicle (Lucy et al., 1991a; Petit et al., 2002). No such effect was detected in the present study. No differences between FM or C supplemented cows were found in follicular size on d 35 or d 37 or growth in diameter from d 35 to d 37. Two possibilities exist for the discrepancy. First, large energy reserves as indicated by body fat (body condition) of the cows in the present study may have masked any effects of supplementation which may be more easily seen in lactating cows. Second, all but one of the studies in which follicular dynamics were altered by feeding omega-3 fatty acids used supplements with a higher linoleic to linolenic acid ratio than used in the present study. Therefore, differences in the effect of dietary supplementation of long chain fatty acids on follicular mechanisms may be related to body fat reserves (energy status of animals) or fatty acid profile of the supplement.

Progesterone. In this study fatty acid supplementation did not affect serum progesterone. In contrast other researchers reported increased levels of P_4 in beef cattle in response to high fat supplementation. However, this effect was reported only on days 6 and 8 of an induced first postpartum estrous cycle in Simmental cows and only on day 5 of the estrous cycle in Brahman cows fed high fat diets (Hightshoe et al., 1991; Lammoglia et al., 1997). Dietary fish meal increased plasma P_4 and mitigated the response to exogenous $PGF_{2\alpha}$ given to synchronize estrus in dairy cattle during early lactation (Burke et al., 1997). This period represents perhaps the greatest period of negative energy balance and physiological stress for the animal due to the heavy requirements imposed by lactation; such conditions are not often found in beef cows. In the present study no effect of rumen protected fish meal on mean progesterone concentration was found. Differences in P_4 concentrations reported in cycling beef cattle fed high or low fat diets occurred early in the estrous cycle, indicating there may be mechanisms by which long chain polyunsaturated fatty acid supplementation may enhance early luteal cell development or P_4 synthesis. Consistent increases in P_4 concentrations in dairy cattle occurred only in early lactation. Thus, while long chain polyunsaturated fatty acid supplementation may not exhibit an overall effect or an effect on established corpora lutea, there may be a mechanism(s) by which it may enhance P_4 synthesis in heavily lactating cattle.

PGFM. Interest in EPA and DHA supplementation has centered on the ability of these fatty acids to attenuate $PGF_{2\alpha}$ synthesis. Several studies both *in vitro* and *in vivo* have reported EPA and DHA supplementation lowered concentrations of PGFM after challenge (Thatcher et al., 1997, 2001; Mattos et al., 2002, 2004). Beef heifers with low luteal phase progesterone and not receiving fish meal secreted greater amounts of $PGF_{2\alpha}$ than heifers receiving fish meal or heifers with high luteal phase progesterone not receiving fish meal (Wamsley et al., 2005). Brahman cows consuming high fat diets with high concentrations of polyunsaturated fatty acids tended to have

greater peak PGFM concentrations than C cows; although, there was no difference in total concentration as indicated by area under the curve (Lammoglia et al., 1997). Petit et al. (2002) reported cows fed formaldehyde-treated linseed with fish oil tended to have greater concentrations of PGFM than either cows supplemented with formaldehyde-treated linseed or duodenally infused with linseed oil. Results in the present study were mixed. Basal levels of PGFM tended to be lower for supplemented cows, while peak concentrations, the change from basal to peak concentration, and estimates of total prostaglandin output did not differ. At 30, 45 and 60 min after OT challenge FM supplementation decreased PGFM concentration ($P < 0.05$) when comparing means on individual days in this study. These data are in accordance with those of Mattos and co-workers (2002) who reported that PGFM concentration was significantly decreased for fish meal supplemented cows only for the period from 15 min to 60 min after oxytocin injection. Mean PGFM concentration was greater for the first replicate than for all others. This may be due to higher animal stress related to canulation procedures that were not present in the subsequent replications. Due to problems with the catheterization equipment and procedures, all animals in Rep1 were recannulated once. Two cows had blood samples taken from the coccygeal vessels and one was bled via jugular venipuncture and received the oxytocin injection in the coccygeal vessels.

It is important to note that the cows used by Thatcher et al. (1997) and Mattos et al. (2002) were dairy animals in the first half of their lactation curves and were located in Florida; while, those used by Petit et al. (2002) were dairy cows in the latter half of lactation and were located in Canada. Both Lammoglia et al. (1997) and Wamsley et al. (2005) used non-gravid beef females. Nutritional and environmental stresses on those animals used by the latter three researchers would have been less than those on the cows used by Thatcher et al. (1997) and Mattos et al. (2002), which had a higher energy requirement and may have been heat stressed. While it has been shown that certain fatty acids have positive effects unrelated to simple energy supplementation, energy balance cannot

wholly be ignored (Mattos et al., 2002). Dairy cows in early lactation would be more likely to be in an acute negative energy balance than those in the latter half of lactation. Such cattle would be expected to exhibit impaired luteal function. Cows having low P_4 levels after mating have been shown to produce higher levels of $PGF_{2\alpha}$ in response to an acute challenge when compared to cows with normal levels of P_4 (Mann and Lamming, 2001). Thus, their magnitude of response to OT challenge may be greater than cows with normal luteal function, accounting for the differing results reported by other researchers and those from the present study.

Intake may also play a role in affecting PGFM concentration. Although there was no difference in mean intake in the present study some sorting by the cows is thought to have occurred. Due to incomplete data no comparisons may be made between crude fat levels or fatty acid profiles of the original concentrates and those of the orts. However, if the orts from cows receiving the FM supplemented concentrate contained a high level of the FM supplement, then adequate intake of EPA and DHA would be in question, as well as, any conclusions drawn on the effects of EPA and DHA supplementation on reproductive responses in the first experiment.

Experiment 2

Fatty Acid Profile. Supplementation of heifers with EnerGII increased mean circulating levels of EPA and DHA, while no change was detected for arachadonic acid levels. These data are in contrast with previous studies that have indicated supplementation of EPA can decrease synthesis of arachadonic acid metabolites (Barham et al., 2000). However, Howie et al (1992) reported fish oil caused a decrease the ratio of arachadonic acid to EPA in uterine fatty acid composition. Other researchers reported fish meal increased plasma EPA concentration in cows (Burns et al., 2002; 2003). This confirms that supplementation of EPA and DHA results in greater circulatory concentrations and thus availability for cellular functions.

Estrus Expression. Spontaneous occurrence of estrus was not affected by fish meal supplementation to Holstein cows on a Florida dairy; although, it did tend to increase the number of cows detected in induced estrus (Burke et al., 1997). Similarly, in the present study rumen protected fish meal supplementation did not affect the number of heifers detected in estrus after synchronization. Fish meal supplementation has been reported to cause greater synthesis of estrogen which may affect estrus expression (Burke et al., 1997). However, no data was collected to determine estrogen levels in the heifers in this study. It is unknown how supplementation of EPA and DHA might modify the cellular process of estrogen synthesis, or if these changes in estrogen production would be enough to alter estrus expression.

Conception and Pregnancy Rates. In the present study no difference was observed in pregnancy rate between heifers supplemented with rumen protected FM or those not receiving FM. Crossbred heifers receiving supplementary dietary fat in the form of Safflower seeds did not differ in pregnancy rate from heifers on the control diet (Lammoglia et al., 2000). Pregnancy rates did not differ between heifers consuming diets supplemented with Megalac or whole sunflower or cotton seeds and heifers on control diets (Filley et al., 2000; Funston et al., 2002; Cuddy et al., 2002). Conception rates for heifers consuming diets supplemented with cottonseeds, soy hulls, or corn and soybean meal did not differ (Wuenschel et al., 2005). Source of dietary fat supplementation or energy does not appear to affect conception or pregnancy rates in heifers as long as total energy in the diet is adequate. In the present study heifers received adequate amounts of nutrition. Positive reproductive responses observed from supplementation of dietary fat, especially polyunsaturated fatty acids, tend to be seen only in cattle under nutritional, lactational, or environmental stress.

Overall, dietary supplementation of rumen protected fish meal to cows and heifers did not significantly affect reproductive responses in a clear manner. Although, changes in circulating fatty acids did occur, positive reproductive responses were not observed.

Chapter 7: Implications

The purpose of this experiment was to quantify the effects of fish meal supplementation on several factors known to influence reproductive efficiency in the beef female, including ovarian and uterine hormones, ovarian follicular dynamics, estrus expression, and pregnancy rates. No significant differences were observed between animals receiving rumen protected fish meal and those receiving traditional energy supplements.

The review of literature coupled with observations in the present study appear to indicate that some reproductive benefit may be seen in cows under nutritional or environmental stress, dairy cows, or *bos Indicus* cows, although the mechanisms of such action are unclear. Further study is warranted in both the beef and dairy animal, especially during peak lactation or in intemperate environments. However, at the present, simple logistical and economical concerns would tend to discourage the beef producer from utilizing this form of supplementation.

During the intensive trial, a high degree of refusal was observed for animals supplemented with the fish meal product. Despite the addition of an abnormally high level of molasses to the ration, palatability was a problem. In the modern dairy the recommended feeding level of 0.45 kg per head per day is easily masked within the 10 plus kilograms of corn silage or total mixed ration consumed by the animal every day. However, the typical beef cow is not fed a total mixed ration and is rarely supplemented. The logistics of feeding and the added cost of the rumen protected fish meal product, as well as, sufficient carrier is a real concern. This concern is doubled by the possibility that certain animals may not consume the supplement, and that no benefit may be seen even if consumed.

Today's beef industry is highly competitive. Producers must use every advantage at their disposal to increase efficiency and return on investment. While fish meal or long chain fatty acid supplementation may yet prove a benefit to the beef producer, further study is warranted.

Literature Cited

Anderson, E. and B. Little. 1984. The onteogeny of the rat granulosa cell. In: D.O. Toft and R.J. Ryan (Ed.) Proceedings of the Fifth Ovarian Workshop. p.203. Ovarian Workshops, Champaign, IL.

Anderson, K.J., D.G. Lefever, J.S. Brinks, and K.G. Odde. 1991. The use of reproductive tract scoring in beef heifers. Agri-Practice. 12:19-26.

Barham, J.B., M.B. Edens, A.N. Fonteh, M.M. Johnson, L. Easter, and F.H. Chilton. 2000. Addition of eicosapentanoic acid to γ-linolenic acid supplemented diets prevents serum arachadonic acid accumulation in humans. J. Nutr. 130:1925-1931.

Bazer, F.W. and H.M. Johnson. 1991. Type I conceptus interferons: Maternal recognition of pregnancy signals and potential therapeutic agents. Am. J. Reprod. Immunol. 26:19-22.

Bazer, F.W., J.L. Vallet, R.M. Roberts, D.C. Sharp, and W.W. Thatcher. 1986. Role of conceptus secretory products in establishment of pregnancy. J. Reprod. Fert. 76:841-850.

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.

Braden, T.D., F. Gamboni, and G.D. Niswender. 1988. Effects of prostaglandin $F_{2\alpha}$ induced luteolysis on the populations of cells in the ovine corpus luteum. Biol. Reprod. 39:245-253.

Burke, J.M., C.R. Staples, C.A. Risco, R.L. de la Sota, and W.W. Thatcher. 1997. Effect of ruminant grade menhaden fish meal on reproductive and productive performance of lactating dairy cows. J. Dairy Sci. 80:3386-3398.

Burns, P.D., T.E. Engle, M.A. Harris, R.M. Enns, and J.C. Whittier. 2003. Effect of fishmeal supplementation on plasma and endometrial fatty acid composition in non-lactating beef cows. J. Anim. Sci. 81:2840-2846.

Burns, P.D., T.R. Bonnette, T.E. Engle, and J.C. Whittier. 2002. Case Study: Effects of fishmeal supplementation on fertility and plasma (omega-3) fatty acid profiles in primiparous, lactating beef cows. Prof. Anim. Sci. 18:373-379.

Burns, P.D., D. B. Abbey, T.R. Bonnette, M.A. Harris, and J.C. Whittier. 2000. Effects of fishmeal supplementation on bovine endometrial concentrations of n-3 fatty acids in Proceedings, Western Section, American Society of Animal Science 53:392-396.

Buskirk, D.D., D.B. Faulkner, W.L. Hurley, D.J. Kesler, F.A. Ireland, T.G. Nash, J.C. Castree, and J.L. Vicini. 1996. Growth, reproductive performance, mammary development, and milk production of beef heifers as influenced by prepubertal dietary energy and administration of bovine somatotropin. J. Anim. Sci. 74:2649-2662.

Ching, K.C. 2000. Fatty acids in foods and their health implication. Marcel Dekker, Inc. New York, NY.

Ciccioli, N.H., R.P. Wettemann, L.J. Spicer, C.A. Lents, F.J. White, and D.H. Keisler. 2003. Influence of body condition at calving and postpartum nutrition on endocrine function and reproductive performance of primiparous beef cows. J. Anim. Sci. 81:3107-3120.

Cryan, R. 2004. U.S. Dairy: Markets and outlook. Volume 10, Number 1. Available: http://www.csg.org/NR/rdonlyres/ewrepgc7hxqw74ivugaax4giczfpt3qrfmnfzb54u3uwdrcm4tgxoonfubtsct7nl3wlvk3fif7qrqxsc3743wooxyg/Economic+multipliers+US_Outlook_May_2004.pdf Accessed: 2005-05-23.

Cuddy, D.L., J.B. Hall, W.E. Beal, and W.S. Swecker. 2002. Effect of high fat diet on reproduction in replacement beef heifers. J. Anim. Sci. 80(Suppl. 1): 410. (Abstr.)

Cunningham, J. 2002. Textbook of Veterinary Physiology. 3rd ed. pp.380-384. W.B. Saunders, Philadelphia, Pa.

Defrain, J.M., A.R. Hippen, K.F. Kalscheur, and R.S. Patton. 2005. Effects of feeding proprionate and calcium salts of long chain fatty acids on transition dairy cow performance. J. Dairy Sci. 88:983-993.

de Vries, M.J. and R.F. Veerkamp. 2000. Energy balance of dairy cattle in relation to milk production variables and fertility. J. Dairy Sci. 83:62-69.

Diskin, M.G. and J. M. Sreenan. 1980. Fertilization and embryonic mortality rates in beef heifers after artificial insemination. J. Reprod. Fert. 59:463-468.

Drackley, J.K., T.M. Cicela, and D.W. LaCount. 2003. Responses of primiparous and multiparous Holstein cows to additional energy from fat or concentrate during summer. J. Dairy Sci. 86:1306-1314.

Dunn, T.G. 1980. Relationship of nutrition to successful embryo transplantation. Theriogenology. 13:27-39.

Echternkamp, S.E. and W. Hansel. 1973. Concurrent changes in bovine plasma hormone levels prior to and during the first postpartum estrous cycle. J. Dairy Sci. 37:1362-1370.

ERS. 2005. Economic Research Service: United States farm and farm-related employment, 2002. Available: http://www.ers.usda.gov/Data/FarmandRelatedEmployment/ViewData.asp?GeoAreaPick=STAUS_United%20States&YearPick=2002 Accessed: 2005-05-23.

Filley, S.J., H.A. Turner, and F. Stormshak. 2000. Plasma fatty acids, prostaglandin $F_{2\alpha}$ metabolite, and reproductive response in postpartum heifers fed rumen bypass fat. J. Anim. Sci. 78:139-144.

Flint, A.F., E.L. Sheldrick, T.J. McCann, and D.S.C. Jones. 1990. Luteal oxytocin: Characteristics and control of synchronous episodes of oxytocin and $PGF_{2\alpha}$ secretion at luteolysis in ruminants. Domest. Anim. Endocrinol. 7:111-124.

Folch, J., M. Lees, and G.H. Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226:497-509.

Fortune, J.E. 2003. The early stages of follicular development: activation of primordial follicles and growth of preantral follicles. Anim. Reprod. Sci. 78:135-163.

Fritz, M.A., and T.A. Fitz. 1991. The functional microscopic anatomy of the corpus luteum: The small cell-large cell controversy. Clin. Obstet. And Gynecol. 34:144.

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

Funston, R.N., T.W. Geary, R.P. Ansotegui, J.J. Lipsey, M.D. MacNeil, and J.A. Paterson. 2002. Supplementation of whole sunflower seeds before AI in beef heifers. Prof. Anim. Sci. 18:254-257.

Garcia-Bojalil, C.M., C.R. Staples, C.A. Risco, J.D. Savio, and W.W. Thatcher. 1998. Protein degradability and calcium salts of long chain fatty acids in the diets of lactating dairy cows: reproductive responses. J. Dairy Sci. 81:1385-1395.

Galyean, M.L. 1993. Technical Note: An algebraic method for calculating fecal output from a pulse dose of an external marker. J. Anim. Sci. 71:3466-3469.

Ginther, O.J., M.C. Wiltbank, P.M. Fricke, J.R. Gibbons, and K. Kot. 1996. Selection of the dominant follicle in cattle. Biol. Reprod. 55:1187-1194.

Girsh, E., W.Wang, R. Mamluk, F. Arditi, A. Friedman, R.A. Milvae, and R. Meidan. 1996. Regulation of endothelin-1 in the bovine corpus luteum: elevation by prostaglandin F2 alpha. Endocrinology. 137:5191-5196.

Greiner, S. 2001. Genetic relationships. Livestock Update. Available: http://www.ext.vt.edu/news/periodicals/livestock/aps-01_12/aps-0436.html. Accessed August 16, 2006.

Gutierrez, C.G., J. Oldham, T.A. Bramley, J.G. Gong, B.K. Campbell, and R. Webb. 1997. The recruitment of ovarian follicles is enhanced by increased dietary intake in heifers. J. Anim. Sci. 75:1876-1884.

Hall, J.B. 2005. Recommended estrous synchronization systems for heifers. Pages 119-126 in Proc. Applied Reproductive Strategies in Beef Cattle, Lexington, KY.

Hall, J.B., R.B. Staigmiller, R.A. Bellows, R.E. Short, W.M. Moseley, and S.E. Bellows. 1995. Body composition and metabolic profiles associated with puberty in beef heifers. J. Anim. Sci. 73:3409-3420.

Helmer, S.D. P.J. Hansen, W.W. Thatcher, J.W. Johnson, and F.W. Bazer. 1989. Intrauterine infusion of highly enriched bovine trophoblast protein-1 complex exerts an antiluteolytic effect to extend corpus luteum lifespan in cyclic cattle. J. Reprod. Fert. 87:89.

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. Supp.):E90-E106.

Hightshoe, R.B., R.C. Cochran, L.R. Corah, G.H. Kiracofe, D.L. Harmon, and R.C. Perry. 1991. Effects of calcium soaps of fatty acids on postpartum reproductive function in beef cattle. J. Anim. Sci. 69:4097-4103.

Homanics, G.E. and W.J. Silvia. 1988. Effects of progesterone and estradiol-17β on uterine secretion of prostaglandin $F_{2\alpha}$ in response to oxytocin in ovariectomized ewes. Biol. Reprod. 38:804-811.

Howie, A., H.A. Leaver, N.H. Wilson, P.L. Yap, and I.D. Aitken. 1992. The influence of dietary essential fatty acids on uterine C20 and C22 fatty acid composition. Prostaglandins Leukot. Essent. Fatty Acids. 46:111-121.

Hulshof, S.C.J., J.R. Figueiredo, J.F. Beckers, M.M. Bevers, J.A. van der Donk, and R. van den Hurk. 1995. Effects of fetal bovine serum, FSH, and 17β-estradiol on the culture of bovine preantral follicles. Theriogenology. 44:217-226.

Inskeep, E.K. 2004. Preovulatory, postovulatory, and postmaternal recognition effects of concentrations of progesterone on embryonic survival in the cow. J. Anim. Sci. 82(E. Suppl.):E24-E39.

Jenkins, T.G. and C.L. Ferrell. 2002. Beef cow efficiency-revisited. Pages 32-43 in 34th Annual Beef Improvement Federation Proceedings, Omaha, NE.

Johnson, S.K., G.H. Deutscher, and A. Parkhurst. 1988. Relationships of pelvic structure, body measurements, pelvic area, and calving difficulty. J. Anim. Sci. 66:1081-1088.

Kastelic, J.P. 1994. Understanding ovarian follicular development in cattle. Vet. Med. 89:64-71.

Konrad, R.J., J.Z. Stoller, Z.Y. Gao, and B.A. Wolf. 1996. Eicosapentanoic acid (C20:5) augments glucose induced iInsulin secretion from β-TC3 insulinoma cells. Pancreas 13(3):253-258.

Knickerbocker, J.J., W.W. Thatcher, F.W. Bazer, M. Drost, D.H. Barron, K.B. Fincher, and R.M. Roberts. 1986a. Proteins secreted by day 16 to 18 bovine conceptuses extend corpus luteum function in cows. J. Reprod. Fert. 77:381-391.

Knickerbocker, J.J., W.W. Thatcher, F.W. Bazer, D.H. Barron, R.M. Roberts. 1986b. Inhibition of uterine prostaglandin $F_{2\alpha}$ production by bovine conceptus secretory proteins. Prostaglandins 31:777-793.

Kyle, S.D., C.J. Callahan, and R.D. Allrich. 1992. Effect of progesterone on the expression of estrus at the first postpartum ovulation in dairy cattle. J. Dairy Sci. 75:1456-1460.

Lalman, D.L., D.H. Keisler, J.E. Williams, E.J. Scholljegerdes, and D.M. Mallett. 1997. Influence of postpartum weight and body condition change on duration of anestrus by undernourished suckled beef heifers. J. Anim. Sci. 75:2003-2008.

Lammoglia, M.A., R.A. Bellows, E.E. Grings, J.W. Bergman, S.E. Bellows, R.E. Short, D.M. Hallford, and R.D. Randel. 2000. Effects of dietary fat and sire breed on puberty, weight, and reproductive traits of F_1 beef heifers. J. Anim. Sci. 78:2244-2252.

Lammoglia, M.A., S.T. Willard, D.M. Hallford, and R.D. Randel. 1997. Effects of dietary fat on follicular development and circulating concentrations of lipids, insulin, progesterone, estradiol-17β, 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$, and growth hormone in estrous cyclic Brahman cows. J. Anim. Sci. 75:1591-1600.

LeMarie, W.J. 1989. Mechanisms of mammalian ovulation. Steriods 54:455-469.

Lipner, H. 1988. Mechanism of ovulation. In: E. Knobil and J. Neill (Ed.) The physiology of reproduction. pp.447-488. Raven Press, New York.

Louhio, H., O. Hovatta, J. Sjoberg, and T. Tuuri. 2000. The effects of insulin and insulin-like growth factors I and II on human ovarian follicles in long-term culture. Mol. Hum. Reprod. 6: 694-698.

Lucy, M.C., C.R. Staples, W.W. Thatcher, P.S. Erickson, R.M. Cleale, J.L. Firkins, J.H. Clark, M.R. Murphy, and B.O. Brody. 1992a. Influence of diet composition, dry matter intake, milk production and energy balance on time of postpartum ovulation and fertility in dairy cows. Anim. Prod. 54:323.

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

Lucy, M.C., C.R. Staples, F.M. Michel, and W.W. Thatcher. 1991a. Effect of feeding calcium soaps to early postpartum dairy cows on plasma prostaglandin $F_{2\alpha}$, luteinizing hormone, and follicular growth. J. Dairy Sci. 74:483-489.

Lucy, M.C., R.L. de la Sota, C.R. Staples, and W.W. Thatcher. 1991b. Effect of dietary calcium salts of long chain fatty acids (CaLCFA), energy intake, and lactation on ovarian follicular dynamics in Holstein dairy cows. J. Anim. Sci. 69(Suppl. 1):451 (Abstr.).

Mackey, D.R., J.M. Sreenan, J.F. Roche, and M.G. Diskin. 1999. Effect of acute nutritional restriction on incidence of anovulation and periovulatory estradiol and gonadotropin concentrations in beef heifers. Biol. Reprod. 61:1601-1607.

Mann, G.E. and G.E. Lamming. 2001. Relationship between maternal endocrine environment, early embryonic development, and inhibition of luteolytic mechanism in cows. Reproduction 121:175-180.

Mattos, R., C.R. Staples, A. Arteche, M.C. Wiltbank, F.J. Diaz, T.C. Jenkins, and W.W. Thatcher. 2004. The effects of feeding fish oil on uterine secretion of $PGF_{2\alpha}$, milk composition, and metabolic status of periparturient Holstein cows. J. Dairy Sci. 87:921-932.

Mattos, R., C.R. Staples, J. Williams, A. Amorocho, M.A. McGuire, and W.W. Thatcher. 2002. Uterine, ovarian, and production responses of lactating dairy cows to increasing dietary concentrations of Menhaden fish meal. J. Dairy Sci. 85:755-764.

McCracken, J.A., E.E. Custer, J.A. Eldering, and A.G. Robinson. 1996. The central oxytocin pulse generator: A pacemaker for the ovarian cycle. Acta Neurobiol. Exp. (Warsz.) 56:819-832.

McWillimas, D., A.M. Dunn, E. Esquivel, and M.E. Wise. 1998. Direct effects of luteal regression on anterior pituitary response to GnRH. Domest. Anim. Endocrinol. 15:209-215.

Meidan, R., E. Aberdam, and L. Aflalo. 1992. Steriodogenic enzyme content and progesterone induction by cAmp monophosphate generating agents and prostaglandin $F_{2\alpha}$ in bovine theca and granulosa cells luteinized in vitro. Biol Reprod. 46:786-792.

Meidan, R., E.Girsh, O. Blum, and E. Aberdam. 1990. In vitro differentiation of bovine theca and granulosa cells into small and large luteal like cells: morphological and functional characteristics. Biol. Reprod. 43:913-921.

Meier, S., M.A. Trewhella, R.J. Fairclough, and G. Jenkin. 1997. Changes in Uterine Endometrial Phospholipids and Fatty Acids Throughout the Oestrous Cycle and Early Pregnancy in the Ewe. Prostaglandins Leukot. Essent. Fatty Acids. 57:341-349.

Murdoch W.J., and J.L. Cavender. 1987. Mechanisms of ovulation. Adv. Contracept. Delivery Syst. 3:353-366.

Nancarrow, C.D., J. Buckmaster, W. Chamley, R.I. Cox, I.A. Cumming, L. Cummins, J.P. Drinan, J.K. Findlay, J.R. Goding, B.J., Restall, W. Schneider, and G.D. Thorburn. 1973. Hormonal changes around estrous in the cow. J. Reprod. Fertil. 32:320-321.

NCBA. 2004. National Cattlemen's Beef Association: Industry statistics- November, 2004. Available: http://www.beef.org/poliindustrystatistics.aspx Accessed: 2005-05-23.

NCBA. 2005a. National Cattlemen's Beef Association: Beef industry at a glance. Available: http://www.beef.org/polisituationoutlook.aspx Accessed: 2005-05-23.

NCBA. 2005b. National Cattlemen's Beef Association: Beef industry trade facts. Available: http://www.beef.org/NEWSBeefIndustryTradeFacts02-10-052758.aspx Accessed: 2005-05-23.

NRC, 1996. Page 4 in Nutrient Requirements of Beef Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Niswender, G.D., J.L. Juengel, P.J. Silvia, M.K. Rollyson, and E.W. McIntush. 2000. Mechanisms controlling the function and life span of the corpus luteum. Physiol. Rev. 80:1-29.

Padmanabhan, V., F.M. Convey, J.F. Roche, and J.J. Ireland. 1984. Changes in inhibin-like bioactivity in ovulatory and atretic follicles and utero-ovarian venous blood after prostaglandin-induced luteolysis in heifers. Endocrinology 115:1332.

Park, P.W. and R.E. Goins. 1994. In situ preparation of fatty acid methyl esters for analysis of fatty acid composition in foods. J. Food Sci. 59:1262–1266.

Pate, J.L. 1996. Intercellular communication in the bovine corpus luteum. Theriogenology 45:1381-1397.

Pate, J.L. 1994. Cellular components involved in luteolysis. J. Anim. Sci. 72:1884-1890.

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.

Penderson, E.S. 1951. Histogenesis of lutein tissue of the albino rat. Am. J. Anat. 88:397.

Penny, L.A., D. Armstrong, T.A. Bramley, R. Webb, R.A. Collins, and E.D. Watson. 1999. Immune cells and cytokine production in the bovine corpus luteum throughout the oestrous cycle and after induced luteolysis. J. Reprod. Fert. 115:87-96.

Petit, H.V., R.J. Dewhurst, N.D. Scollan, J.G. Proulx, M. Khalid, W. Haresign, H. Twagiramungu, and G.E. Mann. 2002. Milk production and composition, ovarian function, and prostaglandin secretion of dairy cows fed omega-3 fats. J. Dairy Sci. 85:889-899.

Pratt, C.W. and K. Cornely. 2004. Essential biochemistry. J. Wiley & Sons. Hoboken, NJ.

Priedkalns, J., A.F. Weber, and R. Zemjanis. 1968. Qualitative and quantitative, morphological studies of the cells of the membrana granulosa, theca interna, and corpus luteum of the bovine ovary. Z. Zellforsch 85:501-520.

Pursley, J.R., M.O. Mee, and M.C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using $PGF_{2\alpha}$ and GnRH. Theriogenology 44:915-923.

Randel, R.D. 1990. Nutrition and postpartum rebreeding in cattle. J. Anim. Sci. 68:853-862.

Roberts, R.M., S. Xie, and N. Mathialagan. 1996. Maternal recognition of pregnancy. Biol. Reprod. 54:294-302.

Roberts, R.M., J.C. Cross, and D.W. Leaman. 1992. Interferons as hormones of pregnancy. Endocr. Rev. 13:432-452.

Savio, J.D., W.W. Thatcher, L. Badinga, and R.L. de la Sota. 1990. Turnover of dominant ovarian follicles as regulated by progestins and dynamics of LH secretion in cattle. J. Reprod. Fert. Abstr. Ser. 6:23.

Sawyer, H.R., K.d. Niswender, T.D. Braden, and G.D. Niswender. 1990. Nuclear changes in ovine luteal cells in response to $PGF_{2\alpha}$. Domest. Anim. Endocrinol. 7:229-237.

Schallenberger, E., D. Schams, B. Bullermann, and D.L. Walters. 1984. Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during prostaglandin induced regression of the corpus luteum of the cow. J. Reprod. Fert. 71:493-501.

Senger, P.L. 1999. Pathways to pregnancy and parturition. 2nd ed. pp.145-213, 285-303.

Silvia, W.J. and G.D. Niswender. 1984. Maintenance of the corpus luteum of early pregnancy in the ewe. III. Differences between pregnant and non-pregnant ewes in luteal responsiveness to prostaglandin $F_{2\alpha}$. J. Anim. Sci. 59:746-753.

Smith, M.F., E.W. McIntush, and G.W. Smith. 1994. Mechanisms associated with corpus luteum development. J. Anim. Sci. 72:1857-1872.

Sreenan, J.M. and M.G. Diskin. 1983. Early embryonic mortality in the cow: Its relationship with progesterone concentration. Vet. Rec. 112:517-521.

Staples, C.R., J.M. Burke, and W.W. Thatcher. 1997. Influence of supplemental fats on reproductive tissues and performance of lactating cows. J. Dairy Sci. 81:856-871.

Sutphin, T. 2005. The value of estrus synchronization and artificial insemination in the Hillwinds cow herd. Pages 204-208 in Proc. Applied Reproductive Strategies in Beef Cattle, Lexington, KY.

Thatcher, W.W., A. Guzeloglu, R. Mattos, M. Binelli, T.R. Hansen, and J.K. Pru. 2001. Uterine-conceptus interactions and reproductive failure in cattle. Theriogenology 56:1435-1450.

Thatcher, W.W., M. Binelli, J. Burke, C.R. Staples, J.D. Ambrose, and S. Coelho. 1997. Antiluteolytic signals between the conceptus and endometrium. Theriogenelogy. 47:131-140.

Thomas, M.G., B. Bao, and G.L. Williams. 1997. Dietary fats varying in their fatty acid composition differentially influence follicular growth in cows fed isoenergetic diets. J. Anim. Sci. 75:2512-2519.

Trenkle, A. and R.L. Willham. 1977. Beef production efficiency: The efficiency of beef production can be improved by applying knowledge of nutrition and breeding. Science 198:1009-1015.

Van Soest, P.J. 1994. Nutritional ecology of the ruminant. 2nd ed. Cornell University Press, Ithaca.

Walters, D.L. and E. Schallenberger. 1984. Pulsatile secretion of gonadotropins, ovarian steroids and ovarian oxytocin during periovulatory phase of the oestrous cycle in the cow. J. Reprod. Fert. 71:503-512.

Walters, D.L., D. Schams, and E. Schallenberger. 1984. Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during the luteal phase of the oestrous cycle in the cow. J. Reprod. Fert. 71:479-491.

Wamsley, N.E., P.D. Burns, T.E. Engle, and R.M. Enns. 2005. Fish meal supplementation alters uterine prostaglandin $F_{2\alpha}$ synthesis in beef heifers with low luteal phase progesterone. J. Anim. Sci. 83:1832-1838.

Weng, B.C. 2002. Immunomodulation by dietary lipids: Soybean oil, menhaden fish oil, chicken fat, and hydrogenated soybean oil in Japanese quail and Bobwhite quail. Ph.D. Diss., Virginia Polytechnic Institute and State Univ., Blacksburg.

Wuenschel, J.C., D.L. Cuddy, S.P. Greiner, and J.B. Hall. 2005. Effect of source of energy on growth and reproduction of replacement beef heifers. J. Anim. Sci. 83(Suppl. 2):23. (Abstr.)

Zhang, Q., W.X. Wu, P.W. Nathanielsz, and J.T. Brenna. 1995. Distribution of arachadonic, eicosapentanoic, docosahexanoic, and related fatty acids in ovine endometrial phospholipids in late gestation and labor. Prostaglandins Leukot. Essent. Fatty Acids. 53:201-209.

Appendices

APPENDIX I: Experimental Calendar and Procedures, Experiment 1.

Procedure	Calendar Day
Diets Begin group 1	25-Jun
feed/pasture samples	30-Jun
Diets Begin group 2, weigh group2	3-Jul
Diets Begin group 3 weigh group 3 feed/pasture samples	7-Jul
weigh group 1	9-Jul
blood samples group 1	12-Jul
feed/pasture samples	14-Jul
weigh group 2	17-Jul
blood samples group 2	19-Jul
weigh group 3 feed/pasture samples	21-Jul
CIDR, GnRH, blood samples, ultrasound ovaries group 1 weigh group 1	23-Jul
blood samples group 3	24-Jul
feed/pasture samples	28-Jul
$PGF_{2\alpha}$, blood samples, ultrasound ovaries group 1	30-Jul
CIDR, GnRH, blood samples, ultrasound ovaries group 2 weigh group 2	31-Jul
GnRH, blood samples, ultrasound ovaries group 1	1-Aug
blood sample group 1	2-Aug
blood sample group 1	3-Aug
blood sample group 1 CIDR, GnRH, blood samples, ultrasound ovaries group 3 weigh group 3 feed/pasture samples	4-Aug

blood sample group 1	5-Aug
blood sample group 1 weigh group 1	6-Aug
blood sample group 1 $PGF_{2\alpha}$, blood samples, ultrasound ovaries group 2	7-Aug
blood sample group 1	8-Aug
blood sample group 1 GnRH, blood samples, ultrasound ovaries group 2	9-Aug
blood sample group 1 blood sample group 2	10-Aug
blood sample group 1 $PGF_{2\alpha}$, blood samples, ultrasound ovaries group 3 blood sample group 2 feed/pasture samples	11-Aug
blood sample group 1 Ultrasound ovaries, blood sample, weigh group 2	12-Aug
blood sample group 1 blood sample group 2 GnRH, blood samples,ultrasound ovaries group 3	13-Aug
blood sample group 1 blood sample, weigh group 2 blood sample group 3	14-Aug
blood sample group 1 blood sample group 2 blood sample group 3	15-Aug
E-17β, Oxytocin group 1, blood sample, weigh group 1 blood sample group 2 Ultrasound ovaries, blood sample group 3	16-Aug
blood sample group 2 blood sample group 3	17-Aug
blood sample, weigh group 3 blood sample group 2 feed/pasture samples	18-Aug
blood sample group 2 blood sample group 3	19-Aug

blood sample group 2 blood sample group 3	20-Aug
blood sample group 2 blood sample group 3	21-Aug
blood sample group 2 blood sample group 3	22-Aug
blood sample group 2 blood sample group 3	23-Aug
E-17β, Oxytocin, blood sample, weigh group 2 blood sample group 3	24-Aug
blood sample group 3 feed/pasture samples	25-Aug
blood sample group 3	26-Aug
blood sample group 3	27-Aug
E-17β, Oxytocin, blood sample, weigh group 3	28-Aug

APPENDIX II: VPI Custom Mineral #2

Guaranteed Analysis:	
Calcium (Min)	8.50%
Calcium (Max)	10.20%
Phosphorus (Min)	7.90%
Salt (Min)	17.00%
Salt (Max)	20.00%
Magnesium (Min)	12.00%
Potassium (Min)	1.00%
Sulfur (Min)	0.05%
Cobalt (Min)	50ppm
Copper (Min)	300ppm
Iodine (Min)	60ppm
Manganese (Min)	1200ppm
Selenium	60ppm
Zinc (Min)	1300ppm
Vitamin A	100,000IU/lb
Vitamin D-3	25,000IU/lb
Vitamin E	250IU/lb

Ingredients:
Monocalcium Phosphate, Dicalcium Phosphate, Magnesium Oxide, Salt, Magnesium Mica, Calcium Carbonate, Sodium Selenite, Potassium Chloride, Mineral Oil, Zinc Oxide, Copper Sulfate, Manganous Oxide, Zinc Sulfate, Ferrous Sulfate, Manganese Sulfate, Calcium Iodate, Plant Protein Products, Cobalt Sulfate, Synthetic Red Iron Oxide, Vitamin A supplement, Vitamin D-3 Supplement, and Vitamin E Supplement.

Appendix III: EnerGII Fatty Acid Profile

Ener GII Reproduction Formula

Moisture (%)	2.8-4.2
Ash (%)	14.5-17
Fat Mod. Wer. Schmidt (%)	78-80
Fat by Acid Hydrolysis	79-81
Myristic C14:0 (%)	2.0-2.45
Palmitic C16:0 (%)	40-48
Stearic C18:0 (%)	4-6
Oleic C18:1C (%)	28-37
Vaccenic C18:1C (%)	1-1.5
Linoleic (b) C18:2 (%)	7-13
Eicosapentaenoic (a) C20:5 (%)	2-2.5
Docosahexaenoic (a) C22:6 (%)	1.9-2.5

Appendix IV: Experiment 1 Orts

Cow ID	101	105	108	109	114	128	135	143	153	157	159
TRT	C	C		FM	C	FM	FM	FM	C	FM	C
Rep	1	1		3	2	1	3	1	2	3	2
06/25/04											
06/26/04			257.3			198.4		224.3			
06/27/04			728.4								
06/28/04			2030			439.4					
06/29/04			332.1			321.75					
06/30/04			1479			138.85					
07/01/04			1598			462.1					
07/02/04						1602		38.9			
07/03/04					1051.8	360.2					
07/04/04					26.5	534.95					108.05
07/05/04						1495.8			866.4		219.1
07/06/04						887		20.15			
07/07/04						681.8		21.4			43.3
07/08/04						443.1					
07/09/04						232.25					282
07/10/04						47.8					
07/11/04					675.3	72.5			18.2		
07/12/04						533.8				1632.8	
07/13/04						279.8					
07/14/04					368.5	350.4					
07/15/04						163.25				70.1	384.9
07/16/04						154.2					
07/17/04						173.75	516.45				
07/18/04					284.7	569	303.1				
07/19/04						103.3	1151.8				
07/20/04						395.5	1238.8				
07/21/04				21.7		331.5	1724.8			516.05	
07/22/04						310.4	1180.8	114.6			
07/23/04						111.4	764.8			1839.6	
07/24/04						462.35	693.4				
07/25/04						82.4					
07/26/04						207.4	615.4			230.4	
07/27/04						372.4	167.4			222.4	
07/28/04						289.4	207.4			146.4	
07/29/04				110.4		417.4	188.4				
07/30/04						772.4	778.4				
07/31/04						524.4	320.1			187.7	
08/01/04						142.1					
08/02/04				178.5		566.9	124.5	129.8		46.6	
08/03/04				360			460	248.4		37.9	
08/04/04				266.4		549.9		110.3			
08/05/04				70.6		715.4		216.6		38.2	
08/06/04				190		394.4		9.9			69
08/07/04				247.1		745.4	32.7	198		41.8	
08/08/04				473.9		685.4		37.7			
08/09/04				305.2		551.8	107.9	13		57.4	

Cow ID	162	163	168	169	170	180	194	195	196	197
TRT	FM	C	C	C	FM	FM	C	C	FM	FM
Rep	2	3	3	2	1	1	3	2	2	1
06/25/04										
06/26/04						721.4				
06/27/04						88.7				
06/28/04										
06/29/04										
06/30/04						47.75				
07/01/04										
07/02/04					55.4					
07/03/04	102.9									
07/04/04	44.7				63.2					
07/05/04						135				
07/06/04					56.9			31.8		
07/07/04	86.2	110.5			379.8			190.6		
07/08/04					58.7	25.6				
07/09/04	89.4				84.1			40.45		
07/10/04	610.8				52.3					
07/11/04	212.1		205.8							
07/12/04	207									
07/13/04	19.8				694.8			203.8		
07/14/04	110				61.25					
07/15/04	220.2				126.9	27.3				
07/16/04	1554.8									
07/17/04	324.3				47.7				146.95	
07/18/04	1876.8									
07/19/04										
07/20/04	1185.8									
07/21/04	131.6				67.95					
07/22/04	243.4									
07/23/04	679.4									
07/24/04	291.05				79.5					
07/25/04	725.8									
07/26/04	804.8				94.4					
07/27/04	680.4									
07/28/04	339.4					342.4				
07/29/04	949.8									
07/30/04	1207.8				93.4					
07/31/04	346.6				97.8					
08/01/04	374.6									
08/02/04	419.65				340.1					
08/03/04	291.35				284.8					
08/04/04	463.3				176.6				76.6	
08/05/04	168.9				1069.6				145	
08/06/04	1827.4				666.4					
08/07/04	533.5				1027.4				36.9	
08/08/04	581.95				811.4					
08/09/04	407.6				694.4	416			32.35	

Cow ID	198	202	216	218	W82	W92	W93	X64	X76
TRT	C	FM	C	C	C	FM	C	FM	FM
Rep	1	3	3	1	3	2	3	2	3
06/25/04									
06/26/04									
06/27/04									
06/28/04	280.75								
06/29/04									
06/30/04									
07/01/04									
07/02/04									
07/03/04									
07/04/04									
07/05/04									
07/06/04									
07/07/04							1936.2		
07/08/04									
07/09/04									
07/10/04									
07/11/04									
07/12/04									
07/13/04									
07/14/04									
07/15/04									
07/16/04									
07/17/04							84.2		
07/18/04									
07/19/04									38.85
07/20/04									
07/21/04									
07/22/04									
07/23/04									
07/24/04									
07/25/04									
07/26/04									
07/27/04									
07/28/04									
07/29/04									
07/30/04									
07/31/04									
08/01/04								139.1	
08/02/04									
08/03/04									
08/04/04								55.1	
08/05/04									
08/06/04								13.2	
08/07/04		28							
08/08/04									
08/09/04									

Cow ID	101	105	108	109	114	128	135	143	153	157	159
TRT	C	C		FM	C	FM	FM	FM	C	FM	C
Rep	1	1		3	2	1	3	1	2	3	2
08/09/04				305.2		551.8	107.9	13		57.4	
08/10/04				144.8		512.2	121.2			892.4	
08/11/04				171.1	69.7	272.6				135.6	
08/12/04				337.7		554.1		86.2		49.2	
08/13/04				39.4		508.2		97.4		448.7	
08/14/04				73.9		437.5				73.9	
08/15/04				147.8	174.7	900.4				27.7	
08/16/04						281.6		362.9		40.6	
08/17/04				155.7	150.9		248.1			112.2	
08/18/04				200.2			151.1			105	
08/19/04							59.4			69.4	
08/20/04				108.1			69.55			223.7	
08/21/04										200.9	
08/22/04				239.6	90						
08/23/04											
08/24/04				149.9							
08/25/04							207.3			50.9	
08/26/04				282.2							
08/27/04				543.5						59	
08/28/04										92.1	
Sum	0	0	6424.8	4817.7	2892.1	22340.25	11432.8	1929.55	884.6	7648.65	1106.35

Cow ID	162	163	168	169	170	180	194	195	196	197
TRT	FM	C	C	C	FM	FM	C	C	FM	FM
Rep	2	3	3	2	1	1	3	2	2	1
08/10/04	840.6					1106.6				
08/11/04	962.6				740.6				119.3	
08/12/04	1038.6	89.9			792.6				29.6	
08/13/04	258.9				703.4				54.7	
08/14/04	378.9				745.4				66.5	
08/15/04	400.4				490.9				85.4	
08/16/04	350				1129.6				150.3	
08/17/04	437.7								109.3	
08/18/04	527.6								87.85	
08/19/04									29.2	
08/20/04	1135.3								257.6	
08/21/04	1765.3									
08/22/04	1311.3									
08/23/04	395									
08/24/04	332.2									
08/25/04										
08/26/04										
08/27/04										
08/28/04										
Sum	28247.5	200.4	205.8	0	11787.3	2910.75	0	466.65	1427.55	0

Cow ID	198	202	216	218	W82	W92	W93	X64	X76
TRT	C	FM	C	C	C	FM	C	FM	FM
Rep	1	3	3	1	3	2	3	2	3
08/10/04									
08/11/04									
08/12/04		45.7							
08/13/04									
08/14/04									
08/15/04		26.8							
08/16/04		122.2							
08/17/04									
08/18/04									
08/19/04									265.9
08/20/04		83.7							
08/21/04									
08/22/04									
08/23/04									
08/24/04									
08/25/04									
08/26/04									
08/27/04									
08/28/04									
Sum	280.75	306.4	0	0	0	0	2020.4	207.4	304.75

APPENDIX V: Catheterization and Blood Collection Procedures

1. 6ml intravenous injection of 0.5mg/ml estradiol-17β [saline/ethanol (50:50)], 0430 hours.
2. Feed cows after injections are given.
3. Begin canulation procedures, 0600 hours.
    a. Restrain cow in chute, extend neck, and restrain head.
    b. Disinfect neck over the jugular vein using dilute betadine solution.
        i. Wipe area with 4x4 gauze pad soaked in betadine solution.
        ii. Remove solution with clean 4x4 gauze pad.
        iii. Rewipe area with a 4x4 gauze soaked in betadine solution using a clockwise motion starting at the center and moving outward.
        iv. Remove solution with a clean 4x4 gauze pad using a clockwise motion starting at the center and moving outward.
        v. Wipe neck in a longitudinal motion using a 4x4 gauze pad soaked in ethanol.
        vi. Wipe neck in longitudinal motion using clean 4x4 gauze pads until no residue is evident on the pads.
    c. Eclude the jugular vein at the base of the neck.
    d. Using a scalpel, carefully place a 1 inch incision over the jugular vein.
    e. Place a 12 gauge, 1.5 inch, steel hypodermic needle into the vein.
    f. Place 6 inches of a 30 inch section of V7 vinyl IV tubing into the vein through the needle.
    g. Holding the tubing in the vein, remove the needle by sliding it over the tubing.
    h. Insert an 18 gauge leur-lock hub into the protruding end of the tubing.

i. Using a 6cc syringe check the canula by attempting to draw blood.

j. If the canula is working properly inject 2ml of sodium citrate into the canula.

k. Using clean 4x4 gauze pads clean the neck around the incision.

l. Insert tubing through the back of a 4x4 denim pouch.

m. Attach the pouch to the neck of the animal over the incision using livestock glue and/or suture.

n. Coil the excess tubing and place it into the pouch for protection and storage until sampling begins.

o. Place animals into segregated areas for rest until sampling.

    i. Animals should be allowed approximately 30 minutes of rest prior to sampling.

p. Restrain animals in the chute.

q. Sampling begins at 0800 hours (-30min) and follows at 15 minute intervals until 1030 hours (120min).

    i. Two samples will follow at half hour intervals (150 and 180 min).

    ii. The final sample is collected at an hour interval (240min).

r. The collection procedure is as follows:

    i. Withdraw 2ml of blood/sodium citrate solution from the canula and place in waste container.

    ii. Withdraw 10ml of blood from the canula and place in 16x100 collection tubes.

    iii. Inject 1.25ml of sodium citrate into the canula.

s. Following the third blood sample (0min), inject 5ml of oxytocin into the canula after the sample is removed but prior to sodium citrate injection.

t. Following final sample collection remove canulas and pouches. Observe animals to ensure bleeding is stopped.

u. Allow 2 hours clotting time following sample collection prior to centrifugation for 15 minutes at 2000 rpm.

v. Pour serum into 12x75 serum tubes and place in freezer at -20^{o}C.

APPENDIX VI: PGFM Assay

Chemicals and Solutions:

PBS: 1 Liter dd H_20, 9g/L NaCl, 3.89 g $NaH_2PO_4H_2O$ (monobasic 138 MW), 10.22 g Na_2HPO_4 (dibasic, 141 MW), pH 7.0 to 7.2

Gel-PBS (.1%): 1 liter ddH_20, 9 g/L NaCl, 3.89 g $NaH_2PO_4H_2O$ (monobasic 138 MW), 10.22 g Na_2HPO_4 (dibasic, 141 MW), 1 g/L knox unflavored gelatin, pH 7.0 to 7.2

PBS-EDTA: 18.6 g EDTA/L PBS

PGFM Antibody (Ab_1): Obtained from Dr. Bill Silvia, University of Kentucky. Dilute the lyophilized antibody in 5ml Gel PBS. We assumed this to be a 1:10 dilution. Freeze in 200ul aliquots in the -70 freezer. Use a 1:6000 dilution in PBS/EDTA/NRS (1:200) for the assay.

Tritiated PGFM (^{3}H-PGFM): 13, 14-dihydro-15-keto-5, 6, 8, 9,11, 12, 14 (n) – ^{3}H Prostaglandin F_2alpha
(From Amersham – TRK517). Use 10-11ul of ^{3}H per 5 ml of Gel-PBS. Add 100ul of this dilution per assay tube. Ideal CPM are 18000-2000.

Second Ab (Ab_2): Use a 1:10 dilution of SARRG in PBS/EDTA.

PGFM standard: Ordered from Sigma #D4143 (13,14 dihydro-15-ketoprostaglandin F2A). Dilute to 10mg/ml in 100% ethanol. From this solution, make a stock standard in Gel-PBS to a concentration of
2 pg/ul.

Procedures:

Label borosilicate 12x75 glass culture tubes (we purchase ours from Sigma) in triplicate.

Pipette gel-PBS into tubes as indicated on assay sheet (see table below).

Pipette standards, quality controls and unknown samples as indicated on assay sheet.

Pipette 100ul of PGFM AB_1 into all tubes except T and NSB.

Add 100ul of ^{3}H-PGFM to all tubes. Cover tightly with parafilm and vortex approx. 15-30 seconds on low speed.

Incubate 20-24 hours at 4^{o}C.

Add 100ul of Ab_2 to all tubes except T. Cover tightly with parafilm and vortex approx. 15-30 seconds on low speed.

Incubate 44-48 hours at 4^{o}C.

Pour off Procedures:

Turn on centrifuge so it cools down (want it between 6-10^{o} C). Heat a beaker of milli-Q water on a hot plate (set hot plate to level 2).

Add 3 ml of cold PBS to all tubes except total count tubes. Place all tubes except T tubes in black centrifuge racks. All four racks must have equal numbers of tubes or the centrifuge will be off balance. Centrifuge at 3000 g for 30 minutes. If assay is larger than 1 centrifuge load, add cold PBS to all tubes before beginning to spin.

Gently pour off supernatant into tritium waste container and blot tubes on paper towels. Do not pour off T tubes.

Add 1 ml of the heated milli-Q water to all tubes, including the T tubes.

One tube at a time, add 2.5 ml scintillation cocktail and pour into a 7ml scintillation vial.

Rinse tube with 2.5 ml scintillation cocktail. Vortex. Pour into the same scintillation vial.

Let stand overnight in the dark.

Count for 2 min/sample.

Assay Sheet example

ID	**Sample vol. (ul)**	**PAB vol. (ul)**	**1st ab vol. (ul)**	**^{3}H volume (ul)**	**2nd ab vol. (ul)**
T				100	
NSB		700		100	100
Bo		600	100	100	100
5	2.5	597.5	100	100	100
20	10	590	100	100	100
50	25	575	100	100	100
100	50	550	100	100	100
150	75	525	100	100	100
250	125	475	100	100	100
500	250	350	100	100	100
1000	500	100	100	100	100
HC	200	400	100	100	100
LC	200	400	100	100	100
Samples(s)	200	400	100	100	100
HC	200	400	100	100	100
LC	200	400	100	100	100
Bo		600	100	100	100
NSB		700		100	100
T				100	

Quality controls are stored in micro-centrifuge tubes which are in Ziploc bags in the walk in freezer.

Vita

Jeffrey Carl Wuenschel Jr., son of Jeffrey and Nancy Wuenschel Sr. was born January 7th, 1981 in Conneaut, Ohio. He grew up on a small beef and hay farm and was actively involved in 4H. He graduated from Edgewood Senior High School in Ashatabula, Ohio in June of 1999 where he was a team captain and varsity wrestler. He attended Wilmington College located in Wilmington, Ohio were he wrestled in NCAA Division III and was a team captain his senior year. Jeff graduated in May of 2003 with honors from Wilmington College with a Bachelor of Science degree in Agriculture and a minor in mathematics.

In August of 2003 he began his graduate work at the Virginia Polytechnic Institute and State University in the Animal and Poultry Sciences Department studying reproductive physiology under Dr. John B. Hall. Jeff received a graduated teaching assistantship during his tenure at Virginia Tech. He assisted Dr. Bill Beal and Dr. Cynthia Wood with their Introduction to Animal and Poultry Sciences classes, as well as, Dr. Dan Eversole with his Beef Production class. Additionally, he has assisted Dr. Hall with several research and extension projects. Jeff is currently an active member of the American Society of Animal Scientists and is a member of Delta Tau Alpha- the National Agricultural Honor Society, and the Phi Sigma Society- the National Biological Honor Society.

Quod Erat Demonstrandum.