EFFECT OF SUPPLEMENTING ALGAE TO BREEDING AND EARLY GESTATION NULLIPAROUS HEIFERS ON GROWTH AND REPRODUCTION

by

Griffin T. Nicholls

A Thesis

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Master of Science

The Department of Animal Science

West Lafayette, Indiana

May 2020

THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. Jon Schoonmaker, Chair

Department of Animal Science

Dr. Kara Stewart

Department of Animal Science

Dr. Ron Lemenager

Department of Animal Science

Dr. Bethany Funnell

Department of Veterinary Clinical Sciences

Approved by:

Dr. Zoltan Machaty

Dedicated to Kevyn Miller, for whom I owe all of my accomplishments in the agricultural industry and my friends, mentors, and incredibly supportive family

ACKNOWLEDGMENTS

The attainment of my master's degree would not have been possible without the guidance and support of countless people whom have become invaluable assets shaping me into the man I am today. First and foremost, I would like to thank my advisor Dr. Jon Schoonmaker for giving me the opportunity to pursue a master's degree at my alma mater and home away from home Purdue University. I have always admired how you were present out at the farms and never failed to have a great attitude toward helping his students accomplish the task however dirty or labor intensive it may be. Your open-door policy was a true blessing to my graduate experience. Any question I had, big or small, I felt comfortable meeting face-to-face on an almost daily basis. To my committee members Dr. Ron Lemenager & Dr. Kara Stewart, I want to thank you for providing me with years of incredible memories and a wealth of knowledge. Ron, there is no better time in the day than the time I spend in your office talking about anything and everything cattle. I sat in awe, listening to your years of experience and incredible story telling ability. Your character and achievements encompass everything I strive for in my future. Kara, my life would simply not be the same without you. Your passion for teaching and compassion for others is truly admirable. From boss, to professor, and finally a cherished committee member my Purdue experience has been so incredible with you by my side supporting me every step of the way. Your advice and positive attitude even in the worst of times will stick with me for the rest of my life.

Thank you Dr. Emily Taylor for being my biggest fan from the minute I met you. Emily, I would not be typing these words if we hadn't crossed paths. You have pushed me to be the absolute best I can be while understanding how important a true friend is during the graduate school adventure. Your mentorship and friendship will be dear to me forever.

I cannot thank the Beef Unit enough for the support I had throughout my research. Brian DeFreese, I am forever indebted for all the help you provided me throughout my time at Purdue. There is no question that my most valued time, is time at the farm. Soaking up as much of your knowledge and real-life experiences any time you were present. Charlie Huelsenbeck, thank you so much for dealing with me every time something wasn't quite right. I'm sure my name appearing on your phone was not a great sight. You have become such an amazing friend and make my time out at the farm so incredibly fun! To my fellow graduate students, I could not have done this without you. The Graduate Student Association and those who served alongside me sharpened my

communication and organization skills exponentially. I am so grateful for each and every one of you and look forward to a lifelong friendship. This class of graduate students have become my dearest friends and I am so grateful for all the good times we shared. Nate Briggs, my fellow lab mat and our resident Nittany lion. This whole experience has been a rollercoaster, but you have been such an amazing friend, neighbor, and coworker. Sharing experiences and research hours together has been really a blessing.

I would like to devote a special paragraph to my greatest mentor in life Kevyn Miller. Kevyn took me in from a young age and paved the way for any future success in the agricultural industry. Kevyn always managed to maintain the most warming smile even when times were rough. Our years as hoof trimming partners may have been cut short, but in our time together he single-handedly changed my life. I will never be able to express my gratitude for all Kevyn has done for me. You are in my mind always and the invaluable memories we shared will stick with me until it too, is my time and we meet again.

Finally, to my very dear family. The section I have been avoiding because there are no words to describe my gratitude for your unending support and love. These past few years I have battled with balancing loss of loved ones and poor health while maintaining a strong work ethic. I truly could not have finished this chapter in my life without your love and prayer. I cherish every facetime call with my nephews, siblings, and incredible parents. Through the guidance of my loving father I have come to realize the importance of building strong relationships with the people I surround myself with. Your drive, compassion, and service to help others is something I greatly look up to and strive to emulate in my own life. My mother whom has double duty as my best friend and my rock. There isn't a soul on earth who understands and loves me quite the way you do. I love you both with all of my heart and owe you both all of the thanks for raising me to be the man I am today. All my accomplishments were made possible because of your unwavering support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Fat supplementation has potential to improve reproductive performance and increase pregnancy rates in cattle by increasing the energy density of the diet. However, some of the positive effects of fat seem to be influenced by the type of fatty acid fed. Supplementation of omega-3 (n-3) fatty acids increase uptake of n-3 fatty acids into tissue phospholipids and can mitigate immune and inflammatory responses in favor of pregnancy maintenance in cattle. However, n-3 fatty acid supplementation in ruminants has been associated with a decrease in circulating $PGF_{2\alpha}$, which may delay CL regression, extend an animal's time in diestrus, and prevent ovulation. Prostaglandin $F_{2\alpha}$ is a series 2 prostaglandin, synthesized from omega-6 (n-6) fatty acids, which is inhibited by production of series 3 prostaglandins from n-3 fatty acids. Docosohexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) are long-chain n-3 poly-unsaturated fatty acids (PUFA) that have important biological effects on reproduction through their involvement in hormone and series 3 prostaglandin synthesis. Ruminant tissues are naturally almost devoid of n-3 long-chain PUFA, specifically EPA and DHA. Fish oil is the most common ration additive used to provide very long chain n-3 fatty acids to ruminants. However, marine fish do not synthesize n-3 fatty acids; they consume microscopic algae or other algae-consuming fish to obtain n-3 fatty acids. Algae biomass provides a consistent source of DHA and EPA that could be fed to alter hormonal profiles and improve reproduction of beef heifers. Eighty-eight Angus × Simmental heifers (427 ± 1.8 kg) were blocked by BW and allotted to 2 treatments (44/treatment, 4 pens/treatment, 11 heifers/pen). Control heifers were fed a diet that contained (DM basis) 52.8% mixed grass silage, 32% corn silage, and 15.2% concentrate. DHAgold™ (49% fat; 21.8% DHA; DSM Inc.) was included in the algae diet at 1.65% of DM, replacing equal parts of corn and DDGS. Diets were formulated to contain 12% CP and 0.79 Mcal/kg NEg. Heifers were fed treatment diets from 54 d

prior to the breeding season through the first trimester. Follicular fluid was collected on day 47 for hormonal analysis. Artificial insemination (AI) was from d 55 to 98, after which open heifers were removed to 1 control and 1 algae pen and placed with a bull. The study ended on d 180. Performance data were analyzed using the MIXED procedure and conception data were analyzed using the GLIMMIX procedure of SAS. Dominant follicle diameter and follicular estrogen concentration were unaffected by treatment ($P \geq 0.12$). Follicular insulin-like growth factor-1 was greater in algae compared to control heifers ($P=0.03$). During the pre-breeding period, algae supplemented heifers had lesser DMI ($P=0.006$), and greater ADG ($P=0.03$) during the breeding period, while BW tended to be greater compared to control heifers on d 98 and 180 ($P \leq 0.07$). First service conception rate did not differ between treatments ($P=0.67$); however, second service tended ($P=0.08$) and overall conception was ($P=0.03$) lesser in algae compared to control heifers. These data suggest supplementing DHA-rich algae improved growth, but decreased conception rates of primiparous beef females.

CHAPTER 1. REVIEW OF LITERATURE

1.1 Introduction

1.1.1 Beef Production in the United States

Production and management of beef cattle in the United States is diverse and ever-changing. Consumer demand, economic trends, and environmental shifts may account for some of the diversity, but the vastly differing climates from the East to West coast in America require producer adaptability at the local level. Livestock feed ingredients available in one region may be scarce in the next, leading to an industry that focuses on localized management standards, while maintaining efficiency and sustainability.

1.1.2 Replacement Heifer Maintenance and Development

Cow-calf producers in the United States strive to develop high quality replacement heifers while maximizing profitability. This profitability depends largely on the input costs associated with feed, land, facility and equipment resources, animal health, and labor. Replacement heifers represent the genetic pool that will provide a source of future offspring that will either go on to market and bring economic return to the producer, or become replacement breeding stock. Fertility, maintenance of pregnancy, and ease of calving are a few of the building blocks that together make for a successful replacement heifer. Following weaning, replacement heifers are selected from the heifer calf crop and developed into breeding age females by 12 to 15 months of age (Dziuk and Bellows, 1983). It is generally accepted that heifers need to obtain 60-65% of their mature weight entering the breeding season to optimize reproductive performance (Patterson et al., 1992). The producer's nutritional management directly impacts this phase, consequently toeing the line

between under or overfeeding. Proper management of developing replacement heifers and knowing when and how much to feed, has been proven to increase beef cow performance.

1.1.3 Energy requirements

In order to formulate efficient feeding strategies for cows, it is crucial to understand the production cycle and the requirements at each stage. There are three key stages in the production cycle of a beef cow: late gestation, calving to weaning (early lactation, breeding and early gestation), and post-weaning (mid-gestation). In all these phases activity such as grazing, locomotion, and digestion require energy (Hersom et al., 2007). Maintenance is defined as the amount of feed energy intake that will result in no net loss or gain of energy from cow body tissue (Hersom et al., 2007). Maintenance is the primary focus for producers year-round, but stages such as late gestation and early lactation require additional energy. The dynamics of the yearly cycle are such that nutritional environments periodically do not allow animals to meet nutrient and energy requirements and cows lose weight and body condition. Carefully calculated supplementation is especially necessary late in gestation and through the early periods of lactation. Animals repartition energy at parturition to aid in the final developmental stages of the fetus and the production of milk. Supplementation strategies limit body tissue mobilization by adjusting forage and nutrient intake to ensure requirements are met for maintenance (Parsons et al., 2002). The producer recommendation for adequate body condition is 5 or greater at breeding (1 to 9 scale) for optimal reproductive success (DeRouen et al., 1994; Lents et al., 2003). During early lactation, the cows are likely to be energy deficient and lose body weight and condition for milk production. (Herdt et al., 2000). First and second calf heifers typically have not reached their mature body size, therefore, additional energy is also needed in order to continue growth. Energy is provided to high producing beef females by formulating dynamic rations adjusted for specific physiological

activities. Energy and protein supplementation in the herd are significant expenses for producers, making alternative feed ingredients worth exploring. Moreover, some by-products have shown enhancements in reproductive efficiency of dams and their heifer offspring.

1.2 Reproductive Hormones

The reproductive physiology of livestock is regulated by a complex interaction between the neural and endocrine systems. The interaction of different hormones and neural responses can be further understood by investigating each of their roles independently. The primary role of the nervous system is to provide translation of stimuli into signaling pathways that bring a physiological change to targeted reproductive organs and tissues. These stimuli are unique and are transduced into specified signals responsible for altering a specific target tissue. Neurohormones act as these signals that translate stimuli into physiological changes and are released into the bloodstream following a stimulus to interact with individual target tissues. Neurohormones play a major role in reproduction and lactation. In the female, hormones involved in reproductive physiology are produced primarily in five key locations: the hypothalamus, pituitary, gonads, uterus, and finally from the developing gravid uterus. Regulation of hormonal secretions and neural signaling are controlled by either a positive or negative feedback mechanism and occurs when adequate metabolic status is achieved. The synchronized series of hormones that are necessary for regular estrus activity, ovulation, and pregnancy starts with gonadotropin releasing hormone (GnRH) secretion from the hypothalamus. Gonadotropin-releasing hormone (GnRH) is a decapeptide first discovered and isolated in the hypothalamus in 1971. Hypothalamic GnRH acts on the anterior pituitary and signals for the secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH; Matsuo et al., 1971). These two gonadotrophins act directly on the ovary to regulate production of estradiol, which is produced by antral follicles; and progesterone, which

is produced by the corpus luteum (CL). During the estrous cycle, estradiol and progesterone can act through a negative feedback mechanism to control GnRH secretion, with the exception during the GnRH surge to induce ovulation where estradiol acts in a positive feedback mechanism. In addition, estradiol and progesterone can act directly on the anterior pituitary to regulate FSH and LH secretion. Inhibin, a hormone produced within the gonads, inhibits the production of FSH, but not LH by the pituitary gland.

1.3 Estrous Cycle

The length of the estrous cycle in cattle is approximately 21 days (Hansel et al., 1973) and can range from 17 to 24 days (Wishart, 1972; Salisbury et al., 1978). Each cycle consists of a long luteal phase (days 1-17) where the cycle is under the influence of progesterone and a shorter follicular phase (days 18-21) where the cycle is under the influence of estrogen. The luteal phase is characterized by the presence of a corpus luteum (CL) on the ovary producing progesterone. The follicular phase is characterized by the lack of a functional CL and the presence of a pre-ovulatory dominant follicle that is responsible for secretion of increasing concentrations of estradiol and inhibin. Inhibin acts on the pituitary to inhibit FSH production which prevents additional follicular growth from initiating. At, or immediately prior to estrus, estradiol concentrations peak (Chenault et al., 1975) resulting in the expression of behavioral estrus. Behavioral estrus marks the onset of sexual receptivity and is identified as day 0 of the estrous cycle. The time of peak estrogen secretion can last 6 to 24 hours and eventually stimulates the pre-ovulatory LH surge (Wettemann et al., 1972; Chenault et al., 1975; Schams et al., 1977). The pre-ovulatory LH surge occurs approximately 2 to 6 hours after the onset of estrus (Chenault et al., 1975; Schams et al., 1977; Britt et al., 1981; Kojima et al., 1991) and results in the ovulation of

the dominant follicle(s) at 24 to 32 hours after the beginning of estrus. Ovulation marks the beginning of the luteal phase of the estrous cycle.

Once ovulation has occurred and the egg is released, the cells on the ovary that made up the ovulatory follicle undergo a change termed luteinization, which occurs under the influence of LH. The presence of LH *in vivo* elevates the concentration of adenylate cyclase along with cyclic AMP (cAMP) and ultimately leads to changes in the granulosa cells surrounding the oocyte (Marsh et al., 1966). Luteinization results in the formation of luteal cells and a CL, which secretes progesterone into circulation. The corpus luteum is an endocrine structure that is made up large luteal cells, a product of growing granulosa and thecal cells (Senger, 2012). Progesterone prepares the uterine endometrium for pregnancy, promotes uterine quiescence, and blocks the ovulation of additional follicles. Peak progesterone production occurs about day 12 of the estrous cycle when the CL is fully mature. Progesterone directly inhibits GnRH secretion as well as LH and FSH release, creating a negative feedback loop that inhibits follicular development and prevents ovulation. However, the inhibition of FSH is not as severe as the effect on LH allowing follicular waves to continue through pregnancy.

The primary signal responsible for maternal recognition of a pregnancy in ruminants is a type-one interferon, interferon tau, secreted from the conceptus prior to implantation (Bazer, 1992; Bazer et al., 1998). Interferons are proteins involved in immunomodulatory, antiviral, and antiluteolytic responses in the body of domesticated ruminant species (Roberts et al., 1992). Interferon tau acts on the uterine endometrial tissue to inhibit the expression of oxytocin receptors. Estrogen bound to its receptor will initiate the production of oxytocin receptors. Oxytocin is always released in a pulsatile manner, but interferon tau will block the production of oxytocin receptors, so the endometrial cells are no longer sensitive to the oxytocin. If there is no bound oxytocin

PGF2α cannot be produced. The cascade of events leads to a down regulation of the eicosanoid, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), which is the hormone responsible for the regression of the corpus luteum (Spencer et al., 2002). If pregnancy is not established, luteolysis of the CL is initiated by uterine release of $PGF_{2\alpha}$ (Inskeep, 1973; Hixon and Hansel, 1974). The first step in luteolysis is a very short-term increase in blood flow, followed by significant decrease in blood flow initiated by the release of endometrial ET-1 and AngII. The vasodilation causes an 80% decrease in blood flow between days 16 and 18 of the estrous cycle (Niswender et al., 1976, Acosta et al., 2002). In a normal estrous cycle, this luteal regression occurs between day 17 and 19. Reduced progesterone concentrations remove the negative inhibition on the hypothalamus and will give way to increased LH pulse frequency and amplitude, which will induce maturation of a new dominant follicle. Prostaglandins have very short half-lives and thus have become a major tool used in the development of reproductive synchronization protocols (Senger, 2012). There is evidence connecting the consumption of omega-3 fatty acid rich diets with a decrease in circulating inflammatory markers such as $PGF_{2\alpha}$ (for a review, see Abayasekara and Wathes, 1999; Mattos et al., 2000). Inflammatory eicosanoids, specifically $PGF_{2\alpha,}$ can significantly impact reproductive outcomes such as the onset of estrus, embryo survival, and parturition. There is a link between diets including omega-3 supplementation and a longer time to reach estrus and subsequent parturition due to the reduction of $PGF_{2\alpha.}$ However, impact on measures of success such as pregnancy rate and effects on future offspring health and production are still largely unknown.

Regression of the CL begins the follicular phase and results in increased release of LH into circulation which acts directly on the dominant follicle to cause maturation and stimulate synthesis and secretion of estradiol (Wettemann et al., 1972; Schallenberger et al., 1984; Walters and Schallenberger, 1984). As LH pulse frequency increases, increasing amounts of estradiol are

released from the preovulatory follicle, resulting in greater systemic circulating concentrations of estradiol (Schallenberger et al., 1984). These increased concentrations of systemic estradiol further amplify LH pulse frequency (Stumpf et al., 1989) increasing estradiol more which serves as the primary stimulus for the onset of estrus (Walters et al., 1984; Stumpf et al., 1989).

1.4 Follicular Development

The first major follicular structure to appear in females are the primordial follicles. These early follicles are located within the ovary and form as the female embryo develops in utero (Hansel et al., 1983). Over the course of the cow's life, these follicles will develop into primary follicles and compete for growth and dominance. Throughout the lifespan of the female, quantitatively, primordial follicles are the most abundant ovarian structures present. It is estimated that a heifer is born with approximately 133,000 primordial ovarian follicles that will gradually be depleted by 15 to 20 years of age (Erickson, 1966). The stimulus that is responsible for the recruitment of primordial follicles into primary follicles that will eventually ovulate is still unknown.

The primordial follicle is made up of a single oocyte surrounded by one layer of squamous granulosa cells. Growth of these follicular structures requires the granulosa cells surrounding the oocyte to proliferate and differentiate. Proliferation of the squamous cells leads to the formation of several new layers of now cuboidal granulosa cells surrounding the oocyte. Though this growth phase stimulus is unknown, it occurs once roughly 40 granulosa cells make up the largest cross section (Braw-Tal et al., 2002). The follicular structure begins to grow and progressively moves to the cortex, or the periphery of the ovary. The next stage of development involves cell differentiation. The theca cells differentiate into two distinct layers, the theca interna, and the theca

externa. This process is made possible when the theca cells are drawn away from the connective tissue of the ovary. The growth during this phase is believed to be increased because of gonadotropins such as LH and FSH (Dempsey et al., 1937). At this phase the zona pellucida is fully developed and surrounds the follicle. Once the follicle attains 2 layers of granulosa cells it is categorized as a secondary follicle (Driancourt, 1991) until it exceeds 6 layers of granulosa cells. Once the follicle exceeds 6 layers of granulosa cells (Braw-Tal and Yossefi, 1997), it is considered a tertiary, vesicular, or antral follicle and is characterized by a fluid-filled antrum (Braw-Tal and Yossefi, 1997; Driancourt, 1991). Development of the follicles into the antral stage is thought to occur without the support of gonadotropin (gonadotrophin-independent) for more than 60 days prior to the start of follicular waves. Antral follicle development occurs in two phases. The first phase occurs at the time the antrum is acquired. Fluid, called antra, is introduced into small pockets between follicular cells and is responsible for the rapid growth of the follicle, which is now double the original size. Entry into the antral phase may not be due to the presence of gonadotropins. The stage that follows is regulated and gonadotrophin-dependent. The growth in the second stage is much more rapid and involves FSH-driven follicular recruitment, dominant follicle selection, and further dominant follicle development (Mihm et al., 2003). Only one or two dominant follicles will progress to ovulation. Dominant antral follicles can reach up to 20 mm in diameter prior to ovulation (Fair, 2003) and are referred to as the preovulatory or Graafian follicle. It is currently unexplained why certain large follicles are capable of growing to dominance and following through to ovulation, while others become atretic and regress.

Follicular development involves growth, regression, and subsequent replacement with new large follicles occurring multiple times during one 21-day cycle. (Smeaton and Robertson, 1971; Matton et al., 1981). The largest among the follicles present at the time of CL removal are not the

follicles that ovulate. There is a dominant follicle, of measureable size (8-10mm), slowly growing (~1.2 mm/day) until the restraint of progesterone is removed, and then it grows quickly until ovulation (>2mm/day).

Developing follicles that enter the antral stage become gonadotropin dependent. Once this change occurs growth patterns are formed, called follicular waves (Savio et al., 1988; Fortune, 1994; Driancourt, 2001). The growth that occurs within these waves are broken into three phases; recruitment, selection, and dominance. In cattle, the initial phase (recruitment) can last between two and three days (Driancourt et al., 2001; Ginther et al., 1996). Initiation of follicular waves and follicular emergence is stimulated by an elevation of FSH secreted into the bloodstream from the pituitary gland (Adams et al., 1992). An increase in circulating FSH is positively correlated with the growth of a small cohort follicles less than 5mm in diameter (Roche et al., 1998). The total number of follicles that are viable from the elevated FSH stimulation varies based on cow (Ireland et al., 2007). Theca cells present in the newly formed follicles have LH dependence and the granulosa cells have FSH dependence (Ireland and Roche, 1983). During the second phase of the follicular wave (selection) a single follicle is selected from the pool of recruited cohorts to become dominant. Dominant follicles require attainment of LH receptors and is around 8.5mm in diameter (Ginther et al., 1996). This dominant follicle relies on LH for further growth and maturation. The transition from recruitment into selection can be identified by the reduction in circulating FSH. This downregulation of FSH concentration is due to negative feedback from hormones produced by the large follicles, estradiol with negative feedback on the hypothalamus and inhibin's negative feedback on the pituitary (Adams et al., 1992; Gibbons et al., 1997). It is possible for a cow to have multiple dominant follicles; in which case the dam may become pregnant with multiple offspring. The exact mechanism responsible for the selection of a dominant follicle is unknown;

however, IGF-1 activity may play a role in selection and regulation of follicular dominance. Spicer et al., (1993) observed that IGF-1 increased aromatase activity of granulosa cells in vitro. Aromatase, a major player in sexual behavior, is responsible for the conversion of circulating androgens to active estrogenic metabolites in target tissues (Roselli et al., 2007). Fortune et al. (2004) observed that greater concentrations of IGF-1 were due to a reduction in IGF binding proteins found on the dominant follicle. Campbell et al. (1995) and Driancourt et al. (2001) showed that, most of the time, the follicle that acquires LH receptors on the granulosa cells earliest, will become dominant. The remaining cohort of follicles that fail to reach dominance will undergo atresia leading to a reduction in diameter visible by rectal ultrasonography.

1.5 Fetal Development

Four days post-ovulation, the newly formed embryo will enter the uterus of the cow. The next step is maternal recognition, which occurs between days 15 and 18. Once recognition occurs, the initial stages of placentation begin. Ruminant animals are equipped with a cotyledonary placenta which is made up of three main structures. The first structure, the caruncle, is present on the maternal side of the uterine endometrium. The second structure, the cotyledon, is located on the fetal placenta. Together the structures attach to form a placentome, attached with microscopic villi. In most ruminants, placentomes are numbered between 75 and 125 during pregnancy. The placentome structure is the site of physiological exchange between mother and fetus. The placenta determines the metabolic needs for the fetus to survive (Metcalfe et al., 1988; Ferrell, 1989; Reynolds and Redmer, 1995). Nutrients, gases, and waste are transported through the uteroplacental connection (Reynolds and Redmer, 1995, 2001). If the uteroplacental connection is not fully developed, the effects may be determinantal to the growth and development of the calf.

The placental vascular system develops in the earliest stages of fetal growth (Reynolds and Redmer, 1995). The exchange of nutrients between cow and calf is only possible if the placental vasculature has been properly developed. Uterine blood flow impacts the development of the offspring and increases exponentially as the fetus grows. The flow of umbilical exchange follows a similar pattern as the transport of water and oxygen increases during fetal development. The process of increasing the vascularity of caruncles occurs around day 90 of gestation. By day 120 there is a sharp increase in blood flow exchange and density of the vasculature (Ford et al.,1995). This exponential expansion of vascularity is necessary to support the rapidly growing fetus through the final trimester (Reynolds and Redmer, 2001). There are many causes of early embryo loss and abortion, but dam undernutrition is a major component (Diskin et al., 2011). If the dam is not properly meeting her nutritional demands during the development of the vascular attachment, the fetus will be unable to gain the nutrients and oxygen necessary for survival.

Around day 21 or 22 of gestation, a heartbeat can be observed in the developing fetus. This is followed by limb, brain, lungs, and kidneys develop on d 25 (Hubbert et al., 1972). On day 45, the testicles will begin to form in male calves, but descent into the scrotum will not occur until the fifth month of gestation. Female ovarian development occurs several days later around day 50-60. Nearly 75% of fetal growth by weight occurs in the final two months of gestation, or the final trimester (Robinson et al., 1977). In the event of undernutrition, or a rapid shift in diet, this schedule for development is subject to change. Grazul-Bilska (2009) reported that when ewes were limit-fed at 60% of the National Research Council requirements (NRC, 1996), their female offspring had improper reproductive function. Specifically, there was a significant decrease in proliferation of primordial follicles in fetal ovaries collected from restricted dams on d 135 of gestation. Corah (1975) reported that first-calf Hereford heifers fed 65% of their requirements for

100 days prepartum lost weight compared to control cows who received diets that met 100% of their energy requirements based on the NRC recommendations. Heifers on a restricted diet reached puberty on average 19 days later and the calves from restricted dams were significantly lighter and had more deaths reported at or immediately following parturition when compared to the control animals (Corah et al., 1975).

1.6 Effect of nutrition on reproduction

Reproductive efficiency in livestock is dependent on the amount of energy that is available to the animal. Energy requirements for basal metabolism, activity, growth, energy reserves and lactation must be met before cyclicity can be reestablished (Short et al., 1988). Feeding cows high energy diets from calving until breeding decrease the postpartum interval and increase pregnancy rates compared to cows fed adequate energy diets (Wiltbank et al., 1964; Dunn et al., 1969). Dietary energy is primarily derived from carbohydrates. However, fat and protein can be significant sources of supplemental energy for the cow herd. Some by-products have shown enhancements in reproductive efficiency of dams and heifer offspring. Distillers grains from the ethanol industry, in particular, contains significant amounts of protein and fat and when fed to cows at amounts greater than 10-12 pounds per cow enhanced reproductive performance (Gunn et al., 2014; Shee et al., 2018). It seems that a nutritional component within distillers grains, apart from the additional energy, is responsible for the enhanced reproduction.

Vonnahme et al. (2012) reported that protein supplementation in cows from day 190 through parturition significantly increases uterine blood flow when compared to non-supplemented animals. Serum IGF-1 concentrations have also been increased when high protein diets are fed (Micke et al., 2010). Increases of blood flow and IGF-1 in circulation may increase

progeny performance. Feeding heifers, a high-protein diet (15.6% CP) during the second trimester resulted in faster growth rates and increased body weights in their offspring at 6 months of age when compared to calves from non-supplemented (5.9% CP) heifers (Miguel-Pacheco et al., 2016). Energy and protein supplementation in the herd are significant expenses for producers, making alternative feed ingredients worth exploring. Although protein supplementation in excess of requirements can be converted into energy, the high price of supplementing protein makes fat a more cost effective alternative (Herd et al., 1986).

Supplementation of fat to cattle is common because of its high caloric density that adds energy to the diet. The addition of fat to the ration increases energy intake, resulting in improved reproduction when the cow enters critical phases of their production requiring higher energy. This is particularly the case for cows entering phases of reproduction (late gestation and early lactation) since it is a low priority for partitioned nutrients (Short et al., 1988). In a review, Staples et al. (1998) concluded that supplementation of 3% of the diet DM as fat has often had a positive influence on reproduction in ruminants. Supplemental fat in the diet may partially alleviate negative energy status following calving. However, the positive impacts that have been observed may occur independent of energy status. The chain length, degree of unsaturation and position of the double bonds in the acyl chain of FA impacts their function and could alter the reproductive mechanisms in ruminants (Staples et al. 1998; Mattos et al. 2000), although the exact mechanisms are still unclear. Potential mechanisms include altered follicle development (Staples and Thatcher 2005), increased concentrations of circulating progesterone (Staples et al. 1998), suppressed luteolytic signals around maternal recognition of pregnancy (Mattos et al. 2000), and improved embryo quality (Cerri et al. 2004). Furthermore, when certain fatty acids are supplemented in a cow ration, they later serve as precursors to important reproductive hormones. An example of this

would be an increase in the availability of fatty acid precursors to increase secretion of steroids and eicosanoids. The alteration of steroid and eicosanoid production has a direct impact on ovarian and uterine function (Mattos et. al 2000). Arachidonic acid, for example, acts as a precursor to $PGF_{2\alpha,}$ a reproductive hormone necessary for ovulation, estrus, embryo survival, and parturition (Hess et al., 2008). Likewise, cholesterol, a precursor to progesterone in all mammals, increases when feeding high levels of fat (Santos et al., 2008). Because progesterone inhibits estradiol secretion from the follicle, estradiol concentrations can also be inhibited by adding fat supplementation to the diet. For example, Oldick et al. (1997) demonstrated that monounsaturated fatty acid infusions into the abomasum increased progesterone concentrations in lactating dairy cows, which led to a decrease in the production of estrogen. This relationship leads to the desensitization of the CL to uterine PGF2α thereby inhibiting luteolysis.

1.7 Introduction to fatty acids

Saturated fatty acids do not have any carbon-carbon double bonds, while unsaturated fatty acids have at least one carbon-carbon double bond. A fatty acid (FA) with a single carbon-carbon double bond is categorized as a monounsaturated fatty acid. When a fatty acid contains two or more carbon-carbon double bonds it is a polyunsaturated fatty acid (PUFA; Morgan and Scrimgeour, 1994). Unsaturated fatty acids are typically derived from the green leaf portion of forages. Linoleic acid is an 18-carbon omega-6 fatty acid with 2 double bonds in its structure and is predominately found in grains. Soybean and sunflower oil are known to be high in linoleic acid. Linolenic acid is an 18-carbon omega-3 fatty acid with 3 double bonds in its structure and is found primarily in forages, certain nuts, vegetables and fish oils. Omega-3 fatty acids are characterized by the presence of a double bond three atoms away from the terminal methyl group in their chemical structure, whereas omega-6 fatty acids are characterized by the presence of a double bond

six atoms away from the terminal methyl group in their chemical structure. During rumination, microbes saturate the double bonds in unsaturated fatty acids (biohydrogenation) decreasing the amount of dietary unsaturated fatty acids reaching the animal's downstream absorption and ultimately circulation. The degree to which biohydrogenation occurs is dependent on the type and the quantity of fat entering the rumen (Gulati et al., 1997). Polyunsaturated FAs are particularly affected during the process of biohydrogenation (Demeyer and Doreau, 1999).

Essential fatty acids are not synthesized in the body and therefore must be obtained from the animal's diet. Linoleic acid (LA, 18:2 n-6) and alpha-linolenic acid (ALA 18:3 n-3) are PUFA's that are considered essential because they are required for phospholipid synthesis and are responsible for the production of prostaglandins and steroid precursors. These essential fatty acids also play a major role in anti-inflammatory responses and regulation of neuronal firing (Kapalka, 2010). Prostaglandins are a group of biologically active signaling molecules called eicosanoids that have diverse hormone-like effects in the body. Eicosanoids are synthesized from 20-carbon unsaturated fatty acids (Wolfe et al., 1982). Twenty carbon fatty acids are created from elongation of the essential fatty acids 18:2 and 18:3 (Figure 1.1). Two-series prostaglandins including PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and thromboxane A_2 (TxA_2) are derived from arachidonic acid (20:4n6), which is a 20 carbon omega-6 fatty acid that is elongated from linoleic acid (18:2n6). Prostaglandins derived from eicosapentaenoic acid (EPA, 20:5n-3) are considered "3-series" PGs and include PGD_3, PGE_3, $PGF_{3\alpha}$, PGI_3, and TxA_3 (Figure 1.1). The effect of 3-series vs 2-series PG on reproduction is unknown. However, research has determined 2 and 3-series prostaglandins are heavily involved in immune response, blood pressure regulation, as well as gut health and gastrointestinal integrity.

Modifying PUFA intake can alter the synthesis and metabolism of prostaglandins (for a review, see Abayasekara and Wathes, 1999; Mattos et al., 2000). Elongation of both n-6 and n-3 fatty acids require the enzyme Δ-6 desaturase, making essential fatty acids competitive inhibitors of each other. Though competitive, Δ-6 desaturase has a higher affinity for n-3 fatty acids (Mattos et al., 2000). Thus, changing the intake of n-6 and n-3 fatty acids can alter the balance of 2 and 3 series prostaglandins produced (Chagas et al., 2007). Dietary supplements rich in n-3 PUFA reduce the concentrations of 2-series PG and increase the synthesis of 3-series PG, which are believed to be less inflammatory. An increase in dietary linolenic acid decreases circulating arachidonic acid (Mattos et al., 2000), the precursor for PGF2α. Synthesis of eicosapentaenoic acid (EPA), the precursor of 3-series prostaglandins, increases as a result of increased levels of linolenic acid. The inhibition of prostaglandin synthesis is due to the prior down regulation of phospholipase A2 and cyclo-oxygenase (Thatcher et al., 1995). Linoleic acid and eicosapentaenoic acid are identified inhibitors of cyclooxygenase located in endometrial tissue of ruminants which leads to the suppression of $PGF_{2\alpha}$ secretion. In theory this suppression could decrease the occurrence of early embryonic loss. Furthermore, when fed in excess, other long chain and n-6 fatty acids can inhibit further elongation and desaturation of linoleic and linolenic acids to produce 20 carbon fatty acids/eicosanoids (Palmquist et al., 2010). In vitro studies have demonstrated that n-3 and n-6 fatty acids have an inhibitory effect on oxidation (enzymatic or non-enzymatic) of arachidonic acid or other 20 carbon fatty acids to eicosanoids (Levine and Worth 1994; Archard et al., 1997). Eicosapentaenoic (EPA; 20:5, n-3) can also be elongated into docosahexaenoic acid (DHA; 22:6, n-3). In vitro studies have revealed that although EPA concentrations increase when DHA is present, this increase is due to a sparing of EPA from metabolism into other products rather than a retro-conversion of DHA into EPA (Metherel et al., 2019)

1.8 Effect of dietary fat on follicular development

Multiple studies have been conducted evaluating different forms of fat supplementation and their effect on follicular dynamics. Oldick et al. (1997) observed that dairy cows ruminally infused with fat (yellow grease, high in linoleic acid or tallow), but not glucose, had decreased plasma estradiol concentrations and increased plasma progesterone concentrations compared to cows only infused with water. Corpus luteum diameter was not affected by the fat infusion treatments; however, subsequent infusions of yellow grease down-regulated PGF2α secretion, possibly because the enzyme that catalyzes the synthesis of prostaglandin from arachidonic acid, prostaglandin endoperoxide synthase (PES), was inhibited by the high linoleic acid content of the grease (Oldick et al., 1991). Dietary supplementation of calcium salt of palm oil (2.2% of diet DM) for the first 60 d post-partum has been shown to increase follicle size and quality in post-partum dairy cows (Lucy et al., 1991). Hightshoe et al. (1991) observed that grazing Simmental cows supplemented with 0.5% of BW of calcium soaps of fatty acids from parturition until second ovulation had lower circulating estradiol compared to cows fed an isonitrogenous and isocaloric diet. The treatment supplement was high in both palmitic and stearic saturated fatty acids (from Megalac) and was able to eliminate premature regression of the CL (first short cycle). This is beneficial because a shortened luteal phase in cattle, commonly observed as a result of premature exposure to PGF2α, makes pregnancy during that estrous cycle improbable. Hightshoe et al. (1991) also reported that the number of 6-9 mm and 10-15 mm follicles were significantly greater in cows supplemented with calcium salts of fatty acids. Further blood analysis revealed that cholesterol was greater in cattle supplemented with Megalac, but plasma concentrations of progesterone and triglyceride were not altered, indicating that hypercholesteremia as a result of lipid intake was responsible for the increased luteal phase and increased follicle number (Hightshoe et al., 1991).

Luteinizing hormone is responsible for the regulation of luteal activity (Williams et al., 1989). Conversion of cholesterol to progesterone and pregnenolone are stimulated by binding of LH to its receptor on the luteal cells. (Senger et al., 2012). Cholesterol also serves as a precursor for the synthesis of progesterone by ovarian luteal cells. Dietary fat supplementation increases circulating concentrations of cholesterol (Staples and Thatcher, 2005) and progesterone and lengthens the lifespan of the CL in cattle (Williams and Stanko, 2000). A longer CL lifespan could help maintain pregnancy or alternatively, inhibit development of a new follicle if a pregnancy is not established. The main source of cholesterol for luteal cell progesterone synthesis is present in the blood (Armstrong et al., 1970; Bolte' et al., 1974; Lussier-Cacan et al., 1977; Christie et al., 1979). Thus, changes in the type and/or quantity of cholesterol present in the serum could play a major role in the regulation of steroid hormone biosynthesis of the ovary (Henderson et al., 1981). In a three-week study where multiparous beef cows were supplemented with rice bran, a rich source of PUFA, to the diet (5.20%), researchers observed an increase in wave amplitude in the ovulatory stage of estrous compared to cows fed a 3.74% fat diet (Lammoglia et al., 1997). The high fat treatment also caused a significant increase in serum insulin and cholesterol concentrations (Lammoglia et al., 1997). Wehrman et al. (1991) observed that lactating Brahman crossbred cows fed a diet with 8% lipid from whole cottonseed for the first 19-21 d post-partum had a greater number of medium follicles (3.1-9.9 mm) when compared to cows fed a diet with 2.5% fat; but diet had no effect on large follicles (≥10 mm). The follicular fluid from cows fed 8% lipid diets contained greater cholesterol when compared to follicular fluid from cows fed 2.5% lipid. In a separate experiment, Wehrman et al. (1991) fed cycling crossbred Brahman heifers (14-16 mo.) at various stages of estrous a diet with 6% fat for 35 d and observed 1.8 times greater blood cholesterol compared to heifers fed a 2.2% fat diet. At the conclusion of the 35-d feeding trial the

heifers were synchronized using PGF2α in order to initiate regression of the CL and stimulate growth of new follicles. Sexual behavior was observed, and once estrus was detected the heifers were ovariectomized for further follicular analysis. Although serum progesterone did not differ between treatments, granulosa cells isolated from ovariectomized heifers fed the 6% fat had greater quantities of pregnenolone (P5) and progesterone (P4) when compared to the granulosa cells from heifers fed 2.2% fat. Heifers fed 1.4 - 1.6 kg of whole cottonseed for 30 d prior to breeding had increased levels of serum cholesterol concentrations than those fed no cottonseed (Wehrman et al., 1991).

It seems that the type of fatty acid present in supplemental fat is important. Zachut et al. (2008) conducted a study feeding either high or low levels of unsaturated fatty acids to dairy cows starting at 256 d of pregnancy until 100 d postpartum and observed that as dietary unsaturated fatty acids increased, the size of preovulatory follicles and concentration of steroid hormones present in the follicles increased, which could ultimately be beneficial to ovarian function. In a follow up study, Zachut et al. (2010) discovered that the oocyte cleavage rate was greater in cows fed an increased ratio of n-3:n-6 fatty acids (as flaxseed oil) from 114 to 208 days in milk compared to the cows fed no supplemental fat, indicating that n-3 fatty acids may be beneficial for oocyte development. The ovulation of larger follicles may result in the formation of larger corpora lutea with increased steroidogenic capacity resulting in greater progesterone production, which has been associated with higher conception rates. The consumption of polyunsaturated plant oils also increases basal serum insulin concentrations in both dairy (Palmquist and Moser, 1983) and beef cows (Ryan et al., 1995; Thomas et al., 1997). It is possible that increased serum concentrations of insulin occurring in response to polyunsaturated fat play a role in mediating increased follicular growth, either directly through its own receptor or indirectly by modulating granulosa cell IGF-I

production. Both Insulin and IGF-1 increase follicular steroidogenesis as the follicles grow and develop (Webb et al., 1992; Spicer and Echternkamp, 1995 et al., Wathes et al., 1995). Greater plasma concentrations of IGF-1 lead to earlier follicular competence and a decrease in postpartum interval (Beam et al., 1997). Beam et al. (1997) observed an increase in IGF-1 and overall fertility when multiparous dairy cows were fed increasing concentrations of fish oil. However, DMI was decreased when the greatest concentration of fish oil (4.15%) was fed, suggesting that poor palatability may be a problem when fish oil is supplemented in excess (Beam et al., 1997). Robinson et al. (2002) reported that cholesterol and IGF-1 were increased in first lactation Holstein cattle fed n-6 PUFAs, but not in cows fed n-3 PUFAs; however, the n-3 rich fat supplement increased circulating estradiol.

Childs et al. (2008a) observed that when increasing amounts of n-3 rich fish oil (0, 1.04, 2.08 and 4.15% of total DM) were fed to nulliparous beef heifers, that plasma, follicular, and endometrial concentrations of EPA, n-3, and n-6 fatty acids increased linearly. In the follicular fluid, the increased EPA from the fish oil supplementation led to an overall decrease in the n-6:n-3 PUFA ratio. However, linoleic acid concentrations in the rumen fluid, endometrial tissue, and follicular fluid decreased as fish oil supplementation increased and serum P_4 and E_2 were not affected by fish oil (Childs et al., 2008a). In a second study, Childs et al. (2008b) observed that plasma concentrations of arachidonic acid and cholesterol were significantly greater in heifers supplemented with 2% fish oil (high in n-3) or 2% whole raw soybean (high in n-6) compared to heifers not supplemented with fat. The fish oil supplemented cows had higher levels of arachidonic acid than those on the soybean diet, however, increased cholesterol from fat supplementation did not differ between the two treatments. However, Childs (2008b) reported no effect of fat supplementation on plasma progesterone concentrations or CL diameter. Both experiments were

conducted during the breeding season and used 2% fish oil inclusion as the ideal concentration. Ambrose et al. (2006) observed that supplementing 750 g of rolled flaxseed (56.7% omega-3 ALA) to lactating Holstein cows during the breeding season (28 d prior to and 32 d after AI) tended to increase the size of ovulatory follicles, increased conception rates, and decreased pregnancy loss (27.3 vs 9.8%) compared to cows fed the same amount of rolled sunflower (0.1% ALA) and the control group diet which contained no added fat. However, Thomas et al. (1997) reported that nonlactating beef cows supplemented for 50 d with n-6 rich soybean oil (4% of diet DM) had increased medium sized follicles compared to cows supplemented with n-3 rich fish oil (4% of diet DM). Supplementation of soybean oil and fish oil increased HDL-cholesterol, GH, and follicular fluid IGF-1 when compared to control cattle receiving no fat supplementation. Cows receiving the fish oil supplementation exhibited only marginal changes in follicular growth and markedly delayed rise in serum insulin compared to the soybean oil supplementation and the no fat treatment group (Thomas et al., 1997). These observations suggest that the improved follicular growth from n-6 rich soybean oil may be due to the synergistic effects of enhanced insulin release and lipoprotein-mediated changes on the ovary, such as a significant delay in serum insulin in ovaries from the fish oil diets compared to soybean oil diets.

1.9 Fat supplementation on oocyte maturation and quality

Bilby et al. (2006) observed that supplementing 1.35% of the diet DM as mono or poly-unsaturated fatty acids for 15 weeks postpartum did not impact oocyte quality in lactating dairy cows, but cows fed 18:2 or 18:3 enriched diets had larger pre-ovulatory follicles and a subsequent increase in CL size compared to diets formulated for cis and trans 18:1 fatty acid. The exact mechanism for increased follicle size is unknown; however, cellular membranes contain a large

concentration of lipids and the length of the fatty acid acyl chain, number, and position of double bonds influences the biophysical properties of membranes, altering fluidity and cell proliferation (Zeron et al., 2001). Zeron et al. (2002) observed that, compared to a control diet that excluded a protected PUFA supplement, lambs fed calcium soaps of fish oil (9.6% EPA and 7.8% DHA) had increased composition of long chain fatty acids (7.4 and 12.7%) within their plasma and had increased phospholipids in their cumulus cells, which along with granulosa cells surround the oocyte and participate in maturation and fertilization. In addition, Zeron et al. (2002) reported that the number of Grade I oocytes was increased in lambs fed protected fish oil when compared to the control group, indicating that these oocytes were of better quality, were more adaptable and had a greater survival rate in an adverse environment. Fouladi-Nashta et al. (2007) supplemented a high fat diet of Ca salts of palm oil (800 g/d) to lactating dairy cows and observed that the number of blastocysts was increased compared to the low-fat diet providing 200 g/d of Ca salts of palm oil. In a similar study, Cerri et al. (2009) observed that the number of accessory sperm cells able to attach to the zona pellucida in vitro was greater in ova from cows fed Ca salts of saturated and monounsaturated fatty acids compared to cows fed Ca salts of linoleic acid and trans-octadecenoic (18:1 trans) fatty acids. These studies indicate that the type of fatty acid can alter oocyte maturation and early embryo development. Level of unsaturation (PUFA as compared to MUFA) did not alter oocyte quality or embryo development (Bilby et al., 2006).

Linoleic acid (an n-6 fatty acid) has been identified as the most abundant fatty acid present in the follicular fluid that surrounds the oocyte (Marei et al., 2010) and may play a role in regulation of oocyte maturation. Marei et al., (2010) observed that nuclear maturation and cumulus expansion was inhibited when cultured bovine oocytes were supplemented with 100 μM of LA, which was believed to be in response to the observed increase in PGE_2 concentration and increase in the

PGE_2:PGF2α ratio. Homa and Brown et al. (1992) observed that LA was the only fatty acid analyzed that inhibited breakdown of germinal vesicles and that LA concentrations in bovine follicles decreased as the follicles grew in diameter (34.8 vs 31.1% of total FA for large vs small follicles) suggesting that LA plays a role in oocyte growth and maturation. Increasing concentrations of eicosapentaenoic acid (1, 10, and 100 nM) decreased oocyte lipid content and altered the pattern of lipid droplets in grade 1 and 2 bovine oocytes removed from females at various stages of their estrous cycle (Nikoloff et al., 2019). The 100 nM EPA concentration reduced oocyte maturation rate but had no effect on cumulus expansion or embryo development when compared to the lower levels of EPA supplementation (1 and 10 nM).

1.10 Effect of dietary fat on pregnancy rate

Pregnancy rates have been shown to increase when cows are supplemented with dietary fat (Armstrong et al., 1990, Lopes et al., 2009, Bellows et al., 2001). In a 2-year study with three herds of lactating dairy cattle, Armstrong et al. (1990) reported an increased conception rate in cows supplemented with 0.8 kg/d of fish meal from 7 days postpartum until the next calving season. Supplementing fat also reduced the number of services required per conception (1.62 v. 2.31). Lopes et al. (2009) supplemented 0.1 kg/d of rumen protected PUFAs to lactating primiparous Nellore heifers on two commercial cow-calf operations (n=910) in Brazil from the beginning of estrous synchronization until 28 days post fixed timed artificial insemination and reported a greater conception rate compared to heifers fed a control diet (51.2% vs. 39.6%, respectively). Burke et al. (1997) supplemented multiparous lactating dairy cows with ruminant grade fish meal at 0.7 kg/d from d 25 to 88 d postpartum and noted increased conception rates on one farm, but no effect on conception on the other farm. Lucy et al., (1991) hypothesized that the added linoleic acid in the fatty acid treatment may have improved fertility by providing precursors for increased uterine

production of $PGF_{2\alpha}$. The increased $PGF_{2\alpha}$ secretion would have allowed the animal to return to cycling and would have improved follicular recruitment. In contrast, Lucy et al. (1992) reported a decrease in pregnancy rates and a tendency for an increase in time to first service in lactating Holstein cows when supplemented with calcium salts from long chain fatty acids during the early postpartum period.

Another theory suggests that an increase in fertility from fatty acid supplementation may be from an improved energy balance or from an altered lipoprotein composition in the diet, which would stimulate progesterone secretion (Carroll et al., 1992). Sklan et al. (1994) reported an increase in conception rates of primiparous Holstein heifers fed calcium salts of fatty acids (2.5% of DM) from calving to 120 days in milk (DIM) compared to heifers fed a control diet containing no calcium salts (0% of DM), but observed no significant difference in conception rates in multiparous Holstein cows fed the same treatments. The differences between age groups could not be explained by hormone profile as there were no differences in plasma progesterone or estradiol concentrations. Increased energy content of the diet supplemented with calcium salts (1.82 vs 1.71 Mcal of NE_L/kg of DM) may have increased energy status of the heifers to improve conception.

Petit et al. (2006) observed that supplemental soybean meal (high in n-6 fatty acids) for the first 50 to 120 d after calving in lactating dairy cows decreased CL size compared to supplemental flaxseed (high in n-3 fatty acids) or Megalac (high in n-6 fatty acids). Blood analysis on d 17-21 of the estrous cycle showed that plasma concentration of progesterone was greater in cattle fed the flaxseed diet compared to the other two diets. Though the conception rates between all treatments remained unchanged, embryo mortality was decreased from 15.4 and 8.0% in megalac and soybean fed cows, respectively, to 0% in the flaxseed fed dairy cows (Petit et al., 2006). Silvestre et al. (2011) supplemented Holstein cows with either calcium salts of n-6 rich palm oil or safflower

oil (1.5% of DM) from 30 d pre-partum through 30 d postpartum. From 30 to 120 d postpartum cows received either 1.5% of DM as fish oil or calcium salts of palm oil both are sources high in omega-3 fatty acids. Although pregnancy to artificial insemination at 32 and 60 d following first breeding was not affected by diet, pregnancy loss was lower in cattle supplemented with fish oil (Silvestre et al., 2011). Pregnancy losses at second AI did not differ, but n-3 rich fish oil supplemented cows had greater pregnancy rates compared to cows supplemented with calcium salts of palm oil rich in n-6 fatty acids (Silvestre et al., 2011).

1.11 Algae

World population is estimated to increase to over nine billion by the year 2050 (FAO, 2007). As the population grows, the livestock industry must adapt to meet this need by increasing production efficiency. Changes in climate, water shortages, and public opinions are all obstacles that the industry must adapt to in order to maintain a sustainable output. Research is being conducted to identify new feed ingredients that would increase efficiency of animal growth and development. To reach these goals, new raw materials are being analyzed in order to find some of the best alternatives available. Microalgae production requires far less land than planted feed ingredients making utilization of this resource in livestock diets a potentially viable alternative. The other benefit to microalgae cultivation is the ability to utilize land that would not support mainstream agricultural production. Algae production could be considered environmentally friendly as these microorganisms have the capability to be cultivated in water or non-arable land, eliminating this production from competing with current food production (Popp et al., 2016). The high protein content, fatty acid balance, vitamin composition, and other biologically active compounds make this alternative source of omega-3s a more efficient alternative to traditional

planted crops. Furthermore, this rich source of fatty acids is believed to be most efficiently utilized by ruminants, due to their ability to digest the unprocessed algae cell wall (Gouveia et al., 2008).

Fish oil is the most common ration additive used to provide long chain n-3 fatty acids to ruminants. However, fish oil palatability and odor decrease intake in livestock and creates supplemental limitations for desirable concentrations of n-3 fatty acids (DHA and EPA) in the diet. Fish oil is generally not purified into individual fatty acids because the purification process of fatty acids from the fish oil is complex, time consuming and costly (Belarbi et al., 2000). Fish are unable to synthesize essential fatty acids such as DHA and EPA, but instead must obtain it by consuming algae or other algae consuming fish in their diet. Thus, algae may be an alternative dietary ingredient that can provide long chain n-3 fatty acids to livestock. In fact, more than 30% of the current algal production worldwide is sold as an animal feed (Spolaore et al., 2006). The demand for microalgae cultivation mirrors that of the demand for aquaculture worldwide. As aquaculture is the fastest growing sector of agriculture, and microalgae is a key input most enterprises cultivate their own supply (Richmond et al., 2004). However, cultivation, harvest, and dehydration all require economic input and has thus create a cost hurdle for producers. Despite the high cost of production, microalgae are the most economical source of long-chain n-3 fatty acids for use in human and aquaculture nutrition.

Algae are categorized as autotrophic, which means they use sunlight and carbon dioxide, or heterotrophic, which means they do not need sunlight and can use organic carbon as an energy source. Because heterotrophic species do not require light to grow, they are adaptable and thus are ideal for mass production indoors (Priyadarshani and Rath, 2012). New technology allows for heterotrophic production of algal biomass in bulk fermenters that do not require the lighting and electricity inputs previously needed for autotrophic algae growth (Harel et al., 2002). Algal

biomass produced in these facilities takes up less arable land and provides a consistent, high quality source of n-3 fatty acids. Algae is broken into two types according to size: macroalgae and microalgae. Macroalgae is larger in diameter than microalgae, its microscopic counterpart, and are visible to the unassisted human eye. Macroalgae are multicellular while microalgae are unicellular.

The microalgae species, Arthrospira, thought to have therapeutic effects in human food and animal diets, has been used for human consumption for more than 700 years (Richmond et al., 2004). Industrial-scale culture of microalgae was first seen during the 1960's in Japan. The first species to be cultivated and mass produced in the United States was chlorella (Little et al., 1953). Chlorella is unique in its ability to be cultivated in a photoautotrophic or heterotrophic environment. Research has identified a heterotrophic strain of chlorella with a surplus of chloroplasts, Chlorella Regularis, which rapidly grows in sunlight and in the dark utilizing organic carbon sources (Endo et al., 1974). Chlorella was initially cultivated for use in human health as a tablet-form supplement because it is rich in Beta-1, 3-glucans which is thought to boost the immune system and reduce the amount of lipids present in the blood (Spolaore et al., 2006). For over 35 years now the production of Chlorella has increased primarily for Asian aquaculture feed to enhance flesh color and supply the fish with essential fatty acids.

Schizochytrium is a heterotrophic eukaryote commonly used in commercial production of long chain polyunsaturated fatty acids. The two major PUFAs that are produced by Schizochytrium are docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA; Lippmeier et al., 2009). Schizochytrium has become the most commonly used microalgae due to its high oil content (50-77%). The contents of the oil are greater than 90% triglycerides, rich with DHA and low in cholesterol content. All the strains currently in use for commercial production have gone

through extensive toxicological research and testing dating back to the 1990s when the first strains were identified as efficient sources of DHA (Winwood et al., 2015).

1.12 Algae supplementation in livestock

The depletion of natural resources and arable land used for feedstuff production has driven the interest in alternative feedstuffs to the forefront of livestock nutrition research. Ruminants are prime candidates because of their ability to use non-protein nitrogen present in microalgae (Lum et al., 2013). The unique process of rumination also gives ruminants the ability to digest the cell walls of algal organisms (Altomonte et al., 2018). Polyunsaturated fatty acids from the majority of feeds, including PUFA-rich fish oils, are biohydrogenated in the rumen and have an inhibitory effect on the ruminal fermentation and can result in a build-up of 18:1 isomer (Shingfield et al., 2012; Toral et al., 2012; Szczechowiak et al., 2016). However, PUFA from microalgae are less biohydrogenated compared to fish oil (Cooper et al., 2004). Because of these unique aspects of microalgae, there is a need to investigate the use of microalgae supplementation in livestock to determine if there is any nutritional benefit.

Most research to date has focused on the ability of supplemental microalgae to increase PUFA content of meat and milk, making the products "healthier". For example, Carvalho et al. (2018) reported that feeding 100g/d of *Aurantiochytrium limacinum* microalgae increased DHA and n-3 content of beef and seems to shift energy utilization in tissues from glucose to fat compared to feeding no microalgae; however microalgae decreased dry matter intake, increased days on feed, and increased oxidative flavors in beef. Lambs fed 60 g/kg of Dinophyceae microalgae rich in DHA had a more favorable polyunsaturated:saturated fatty acid ratio present in the meat along with increased level of omega-3 fatty acids that resembles the current diet recommendations for humans. Lambs fed 2% of their diet DM as Schizochytrium microalgae had greater concentrations

of omega-3 fatty acids in their intramuscular fat deposits compared to lambs not fed microalgae (Diaz et al., 2017). However, increased n-3 fatty acids could increase oxidation rates of the meat and lambs fed Schizochytrium microalgae required more days to finish, had decreased ADG, and a decrease in intake when compared to lambs not fed algae (Diaz et al., 2017). Nute et al. (2007) observed that 60 g/kg DM of supplemental microalgae increased PUFA and DHA content of lamb meat and had no effect on growth characteristics compared to lamb meat from animals fed 60 g/kg DM of other DHA-rich feeds such as linseed oil, fish oil, protected lipid supplement. A sensory panel determined that meat color was decreased, abnormal flavors tended to increase, and oxidation rate was increased in lambs supplemented with marine microalgae (Nute et al., 2007). Meale et al., (2014) determined that supplementing increasing amounts (0, 1, 2, and 3% of DM) of Schizochytrium microalgae (DHA Gold) for 105 to 140 d before slaughter linearly increased the quantity of DHA present in all tissue types, decreased the n-6: n-3 ratio in all tissues, and had no effect on growth rate of lambs when diets were balanced for energy and protein.

Microalgae supplementation in non-ruminants seems to have an impact on both growth and fatty acid composition of meat, possibly because the fatty acids enter circulation unaltered. Abril et al. (2003) fed 5 levels of Schizochytrium microalgae (none, 1.169, 2.680, 3.391, and 5.746 kg/d) to barrows for the final 120 days before slaughter and observed that the greatest amount of microalgae increased BW gain compared to control. Ribeiro et al., (2014) fed 74 g/kg Schizochytrium microalgae or a maize-based control diet with no microalgae to broiler chickens from day 21 to day 35 of their lives and observed that broilers who received the microalgae supplementation had improved final body weight and body weight gain as well as greater concentrations of long chain fatty acids in meat compared to the control. However, the sensory

panel determined there was a decrease in meat flavor due to oxidation in muscles from birds fed the greatest concentration of microalgae (Ribiero et al., 2014).

Several studies have reported that microalgae supplementation modifies milk fatty acid composition by increasing omega-3 or CLA content. However, the effect of microalgae on intake, BW, milk yield, and milk composition have been variable, which is likely related to the amount or form the algae was fed. Franklin et al. (1999) observed that supplementing 910 g/d of Schizochytrium marine microalgae to primiparous dairy cows for 6 weeks increased the concentration of CLA and omega-3 fatty acids present in milk fat compared to cows not supplemented with microalgae and had no effect on milk yield. However, milk fat decreased and although growth of the cows was not impacted, cows fed microalgae had decreased dry matter intake, likely due to palatability issues (Franklin et al., 1999). Boeckaert et al. (2008) also observed that PUFA, DHA, and n-3 content of milk was increased, but DMI and milk yield were decreased when Schizochytrium was supplemented to cannulated dairy cows at a rate of 43.0 g/kg of DMI (350 to 900 g/d) inserted into the cannula. In addition, Vanbergue et al. (2018) observed that lactating dairy cows (100 days in milk) fed 156 or 340 g/d of Schizochytrium microalgae (DHA Gold) for 10 weeks produced milk with increased unsaturated fatty acids, DHA, and CLA, but had decreased intake, milk yield, and milk fat % compared to cows fed diets without microalgae and cows fed diets that contained n-3 rich linseed. In contrast, when the microalgae dose was decreased to 100 g/d or less, the impacts on intake and milk yield are negligible. For instance, Moran et al., (2017) fed multiparous lactating dairy cows 100 g/d of DHA-rich Aurantiochytrium limacinum marine microalgae to for 84 d starting at 133 days in milk and reported an increase in PUFA and n-3 content of milk, a marginal increase in body condition, and no effect on intake, BW, milk yield, or health compared to cows not fed microalgae. Vahmani et al. (2013) fed a control, encapsulated

fish oil, or encapsulated microalgae supplemented diet to pre-partum Holstein cows in either pastures or confinement for 125 d beginning 30 days prior to calving. The fish oil and microalgae diets provided 200 g/d of lipid with approximately 100 g from encapsulation lipids and 100 g from fish oil or microalgae lipids. Both the fish oil and marine microalgae increased n-3, DHA, and CLA content of milk compared to the control diet, with marine microalgae increasing them the most. Dry matter intake and milk yield were not affected, but milk fat content was decreased in cows fed the microalgae supplement compared to cows fed the fish oil and control diets (Vahmani et al., 2013).

The idea that feeding microalgae at amounts greater than 100 g/d is consistent with a recent report by Marques et al., (2019) who fed mid-lactation dairy cows increasing amounts (2, 4, and 6 g/kg DM = 45, 90, 130 g/d) of Aurantiochytrium limacinum microalgae for 21 d (latin square). Microalgae supplemented cows had improved milk fatty acid content due to linearly decreased saturated fatty acids and linearly increased PUFAs and CLA compared to the microalgae-free control group. Overall milk yield was not altered by microalgae supplementation; however, dry matter intake, and milk fat and protein % were linearly decreased as microalgae supplementation amount increased, the largest decrease being at the greatest microalgae inclusion amount. A linear increase in ruminal pH and apparent digestibility was also noted (Marques et al., 2019). Improvements in milk DHA and CLA were also observed by Stamey et al. (2012) where increasing amounts of microalgae oil (none, 112, 145, and 224g/d as microalgae biomass or purified oil) were fed to lactating dairy cows (193 days in milk) for 21 d (latin square). When microalgae oil was used, dry matter intake was unaffected and milk yield actually increased (Stamey et al., 2012), suggesting that something other than the oil or fatty acids are negatively impacting performance when microalgae is supplemented to cattle.

There is some indication that changes in milk fatty acid profile from microalgae supplementation can decrease oxidative stability and shelf life of milk. Glover et al., (2012), supplemented 100 g of DHA-rich microalgae protected with inert fat twice daily (200 g/d total) to dairy cows for 112 days and observed an increase in oxidation of butter and milk due to algae supplementation. These oxidative characteristics were due to an increase in unsaturated fatty acids present in the milk of microalgae fed cows.

While several studies have investigated the impact of microalgae supplementation on fatty acid profile of milk, very few have investigated the potential impacts of microalgae on reproduction. Similar to previous studies, Sinedino et al. (2017) observed that feeding 100 g/d of Schizochytrium microalgae containing 10% DHA from 27 to 147 d post-partum in dairy cows increased milk yield and true protein by 1.1 kg/d and 30 g/d, respectively, and decreased fat yield 40 g/d in cows fed algae in comparison to cows not fed algae. In addition, Sinedeno et al. (2017) reported that microalgae supplementation increased resumption of cyclicity (77.6 vs 65.9%) in primiparous cows and increased pregnancy rates at the first AI in primiparous and multiparous cows (47.6 vs 32.8%) compared to control cows fed no algae. These increases led to a reduction in days to pregnancy by 22 days compared to control cattle. Sinedino et al. (2017) also reported that the RTP4 gene, which is associated with placental development, immunomodulation and conceptus elongation was upregulated in cows fed microalgae. Vlcek (2017) fed lactating dairy cows Schizochytrium microalgae at 100 g/cow daily from day 47 to 99 of lactation and reported increased milk DHA and no effect on the size of the pre-ovulatory follicle at first or second synchronized estrus, although the size of the corpus luteum was larger in cows fed microalgae. Follicular fluid analysis of progesterone (P4), estradiol, insulin, NEFA, and cholesterol between the control and algae supplemented cattle did not differ. Till et al. (2020) reported that

supplementing lactating dairy cows with 100 g/d of Schizochytrium microalgae for the first 98 d of lactation had no effect on DMI, milk yield, milk fat content, or plasma concentrations of a $PGF_{2\alpha}$ metabolite, This latter observation suggests that the n-3 fatty acids had no effect on $PGF_{2\alpha}$ synthesis (Till et al., 2020). However, microalgae supplementation in this study did increase long-chain fatty acid, PUFA, n-3, and DHA content of milk. Other n-3 rich oils, such as linseed oil (Dirandeh et al., 2013) and fish oil (Mattos et al., 2004) have decreased the $PGF_{2\alpha}$ metabolite in periparturient dairy cows. Hostens et al. (2011) also reported that 2 kg of Schizochytrium algae supplementation (44 g/d docosahexaenoic acid), replacing concentrate, fed to Holstein cows from 3 weeks pre partum to 12 weeks post-partum increased milk yield, but decreased milk fat yield. While algae supplementation did not affect serum glucose, insulin, β-hydroxybutyric acid (BHBA), urea, cholesterol, NEFA, or IGF-1, feeding algae did decrease glucose and increase BHBA concentrations in follicular fluid. These changes in follicular metabolites would be considered detrimental to oocyte development and would suggest that microalgae supplementation could negatively impact reproduction (Hostens et al., 2011).

Research on the effect of microalgae on reproduction in non-ruminants is sparse but suggests a potential benefit for maintenance of pregnancy. Jacobs et al. (2018) reported that mares fed 0.06 g/kg body weight of Schizochytrium microalgae for 45 d prior to and 15 d after synchronized breeding had greater DHA and total omega-3 fatty acid concentrations in endometrial tissue biopsies and greater relative transcript abundance of trophoblast and endoderm markers in preimplantation embryos compared to mares fed an isocaloric diet that contained no supplemental fatty acid. These results indicate that microalgae supplementation has the potential to increase the quality of maternal uterine tissue fatty acid content providing a more desirable preimplantation environment for the embryo. This shift in gene expression is driven by the

microalgae present in the maternal diet. Posser et al., (2018) fed increasing amounts of Schizochytrium microalgae (0, 3.5,7.0,14.0, and 28.0 g/d) to sows from d 85 of gestation to weaning and observed that microalgae did not change the number of still births and that sows receiving the greatest amount of microalgae farrowed heavier piglets compared to all other microalgae treatments. However, sows fed the greatest concentration of microalgae had the greatest weaning-to-estrus intervals when compared to control.

The well-established benefits of energy and fat supplementation to improve reproductive performance in ruminants has been researched in depth, however finding alternative sources that are both economically efficient and environmentally friendly could prove to benefit producers and consumers alike. Omega-3 fatty acid rich microalgae, Schizochytrium, is one such alternative that previously had been identified as a potential supplementation to enhance reproductive performance in cattle. Research studies feeding microalgae have primarily been conducted on lactating dairy cows. Improved fatty acid profiles in milk, increased conception rates, and increased pregnancy rates were observed. It is unknown what the impact of microalgae supplementation is in nulliparous females or in older beef females. The objective of the current study was to determine the effect of supplementing microalgae to late gestation pregnant replacement beef females through the first trimester of their second pregnancy on growth and reproductive performance. Replacement beef heifers were used in the current study due to the physiological burden that comes with first breeding, calving, and subsequent lactation. Replacement heifers are still growing when they are first bred, this partitioning of nutrients makes them candidates for fat supplementation, as they have the potential to undergo reproductive challenges. The lack of research pertaining to supplementing microalgae to ruminants, specifically beef cattle, opened the door for the design of the current study.

1.13 References

Abayasekara, D. R. E., & Wathes, D. C. (1999). Effects of altering dietary fatty acid composition on prostaglandin synthesis and fertility. *Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA)*, *61*(5), 275–287. https://doi.org/https://doi.org/10.1054/plef.1999.0101

Abril, R., Garrett, J., Zeller, S. G., Sander, W. J., & Mast, R. W. (2003). Safety assessment of DHA-rich microalgae from Schizochytrium sp. Part V: target animal safety/toxicity study in growing swine. *Regulatory Toxicology and Pharmacology*, *37*(1), 73–82. https://doi.org/https://doi.org/10.1016/S0273-2300(02)00030-2

AbuGhazaleh, A. A., Potu, R. B., & Ibrahim, S. (2009). Short communication: The effect of substituting fish oil in dairy cow diets with docosahexaenoic acid-micro algae on milk composition and fatty acids profile. *Journal of Dairy Science*, *92*(12), 6156–6159. https://doi.org/https://doi.org/10.3168/jds.2009-2400

Achard, F., Gilbert, M., Bénistant, C., Slama, S. Ben, Dewitt, D. L., Smith, W. L., & Lagarde, M. (1997). Eicosapentaenoic and Docosahexaenoic Acids Reduce PGH Synthase 1 Expression in Bovine Aortic Endothelial Cells. *Biochemical and Biophysical Research Communications*, *241*(2), 513–518. https://doi.org/https://doi.org/10.1006/bbrc.1997.7848

Acosta, T. J., Yoshizawa, N., Ohtani, M., & Miyamoto, A. (2002). Local Changes in Blood Flow Within the Early and Midcycle Corpus Luteum after Prostaglandin F2α Injection in the Cow1. *Biology of Reproduction*, *66*(3), 651–658. https://doi.org/10.1095/biolreprod66.3.651

Adams, G. P., Matteri, R. L., Kastelic, J. P., Ko, J. C. H., & Ginther, O. J. (1992). Association between surges of follicle-stimulating hormone and the emergence of follicular waves in heifers. *Reproduction*, *94*(1), 177–188. https://doi.org/10.1530/jrf.0.0940177

Altomonte, I., Salari, F., Licitra, R., & Martini, M. (2018). Use of microalgae in ruminant nutrition and implications on milk quality – A review. *Livestock Science*, *214*, 25–35. https://doi.org/https://doi.org/10.1016/j.livsci.2018.05.006

Ambrose, D. J., Kastelic, J. P., Corbett, R., Pitney, P. A., Petit, H. V, Small, J. A., & Zalkovic, P. (2006). Lower Pregnancy Losses in Lactating Dairy Cows Fed a Diet Enriched in α-Linolenic Acid. *Journal of Dairy Science*, *89*(8), 3066–3074. https://doi.org/https://doi.org/10.3168/jds.S0022-0302(06)72581-4

ARMSTRONG, D. T., KNUDSEN, K. A., & MILLER, L. S. (1970). Effects of Prolactin Upon Cholesterol Metabolism and Progesterone Biosynthesis in Corpora Lutea of Rats Hypophysectomized During Pseudopregnancy1. *Endocrinology*, *86*(3), 634–641. https://doi.org/10.1210/endo-86-3-634

Bazer, F. W. (1992). Mediators of Maternal Recognition of Pregnancy in Mammals. *Proceedings of the Society for Experimental Biology and Medicine*, *199*(4), 373–384. https://doi.org/10.3181/00379727-199-43371A

Bazer, F. W., Ott, T. L., & Spencer, T. E. (1998). Maternal recognition of pregnancy: Comparative aspects: A review. *Placenta*, *19*, 375–386. https://doi.org/https://doi.org/10.1016/S0143-4004(98)80055-6

Bellows, R. A., Grings, E. E., Simms, D. D., Geary, T. W., & Bergman, J. W. (2001). Effects of Feeding Supplemental Fat During Gestation to First-Calf Beef Heifers11This research was conducted under a cooperative agreement between USDA, ARS, and the Montana Agric. Exp. Stn. Mention of a proprietary product does not constitute a guarantee or warranty of the product by USDA, Montana Agric. Exp. Stn., or the authors and does not imply its approval to the exclusion of other products that may also be suitable. USDA, Agricultural Research Service, Northern Plains Area, is an equal opportunity/affirmative action employer, and all agency services are available without discrimination. Authors express appreciation to J. D. Bottger, Univ. of Wyoming, for analyses of blood samples obtained in Study 2. *The Professional Animal Scientist*, *17*(2), 81–89. https://doi.org/https://doi.org/10.15232/S1080-7446(15)31602-8

Bilby, T. R., Block, J., do Amaral, B. C., Sa Filho, O., Silvestre, F. T., Hansen, P. J., Staples, C. R., & Thatcher, W. W. (2006). Effects of Dietary Unsaturated Fatty Acids on Oocyte Quality and Follicular Development in Lactating Dairy Cows in Summer. *Journal of Dairy Science*, *89*(10), 3891–3903. https://doi.org/https://doi.org/10.3168/jds.S0022-0302(06)72432-8

BK, C., Scaramuzzi, R. J., & Webb, R. (1995). Control of antral follicle development and selection in sheep and cattle. *Journal of Reproduction and Fertility Supplement*, *49*, 335–350.

Boeckaert, C., Vlaeminck, B., Dijkstra, J., Issa-Zacharia, A., Van Nespen, T., Van Straalen, W., & Fievez, V. (2008). Effect of Dietary Starch or Micro Algae Supplementation on Rumen Fermentation and Milk Fatty Acid Composition of Dairy Cows. *Journal of Dairy Science*, *91*(12), 4714–4727. https://doi.org/https://doi.org/10.3168/jds.2008-1178

Bolté, E., Coudert, S., & Lefebvre, Y. (1974). Steroid production from plasma cholesterol. II. In vivo conversion of plasma cholesterol to ovarian progesterone and adrenal C19 and C21 steroids in humans. *Journal of Clinical Endocrinology and Metabolism*, *38*(3), 394–400. https://doi.org/10.1210/jcem-38-3-394

Braw-Tal, R., & Yossefi, S. (1997). Studies in vivo and in vitro on the initiation of follicle growth in the bovine ovary. *Reproduction*, *109*(1), 165–171. https://doi.org/10.1530/jrf.0.1090165

Braw-Tal, R. (2002). The initiation of follicle growth: the oocyte or the somatic cells? *Molecular and Cellular Endocrinology*, *187*(1), 11–18. https://doi.org/https://doi.org/10.1016/S0303-7207(01)00699-2

Britt, J. H., Cox, N. M., & Stevenson, J. S. (1981). Advances in Reproduction in Dairy Cattle1. *Journal of Dairy Science*, *64*(6), 1378–1402. https://doi.org/https://doi.org/10.3168/jds.S0022-0302(81)82710-5

Bruckmaier, R. M., & Blum, J. W. (1998). Oxytocin Release and Milk Removal in Ruminants. *Journal of Dairy Science*, *81*(4), 939–949. https://doi.org/10.3168/jds.S0022-0302(98)75654-1

Burke, J. M., Staples, C. R., Risco, C. A., De La Sota, R. L., & Thatcher, W. W. (1997). Effect of Ruminant Grade Menhaden Fish Meal on Reproductive and Productive Performance of Lactating Dairy Cows1. *Journal of Dairy Science*, *80*(12), 3386–3398. https://doi.org/https://doi.org/10.3168/jds.S0022-0302(97)76314-8

Carroll, D. J., Hossain, F. R., & Keller, M. R. (1994). Effect of Supplemental Fish Meal on the Lactation and Reproductive Performance of Dairy Cows[1]. *Journal of Dairy Science*, *77*(10), 3058–3072. https://doi.org/10.3168/jds.S0022-0302(94)77248-9

Cerri, R. L. A., Bruno, R. G. S., Chebel, R. C., Galvao, K. N., Rutgliano, H., Juchem, S. O., Thatcher, W. W., Luchini, D., & Santos, J. E. P. (2004). Effect of fat sources differing in fatty acid profile on fertilization rate and embryo quality in lactating dairy cows. *J. Dairy Sci*, *87*(Suppl 1), 297.

Cerri, R. L. A., Juchem, S. O., Chebel, R. C., Rutigliano, H. M., Bruno, R. G. S., Galvão, K. N., Thatcher, W. W., & Santos, J. E. P. (2009). Effect of fat source differing in fatty acid profile on metabolic parameters, fertilization, and embryo quality in high-producing dairy cows. *Journal of Dairy Science*, *92*(4), 1520–1531. https://doi.org/https://doi.org/10.3168/jds.2008-1614

Chaiyabutr, N., Faulkner, A., & Peaker, M. (1983). Effects of exogenous glucose on glucose metabolism in the lactating goat in vivo. *British Journal of Nutrition*, *49*(1), 159–165. https://doi.org/DOI: 10.1079/BJN19830021

Chenault, J. R., Thatcher, W. W., Kalra, P. S., Abrams, R. M., & Wilcox, C. J. (1975). Transitory Changes in Plasma Progestins, Estradiol, and Luteinizing Hormone Approaching Ovulation in the Bovine1, 2. *Journal of Dairy Science*, *58*(5), 709–717. https://doi.org/https://doi.org/10.3168/jds.S0022-0302(75)84632-7

Childs, S., Hennessy, A. A., Sreenan, J. M., Wathes, D. C., Cheng, Z., Stanton, C., Diskin, M. G., & Kenny, D. A. (2008). Effect of level of dietary n-3 polyunsaturated fatty acid supplementation on systemic and tissue fatty acid concentrations and on selected reproductive variables in cattle. *Theriogenology*, *70*(4), 595–611. https://doi.org/https://doi.org/10.1016/j.theriogenology.2008.04.002

CHRISTIE, M. H., STRAUSS III, J. F., & FLICKINGER, G. L. (1979). Effect of Reduced Blood Cholesterol on Sterol and Steroid Metabolism by Rat Luteal Tissue*. *Endocrinology*, *105*(1), 92–98. https://doi.org/10.1210/endo-105-1-92

Cooper, S. L., Sinclair, L. A., Wilkinson, R. G., Hallett, K. G., Enser, M., & Wood, J. D. (2004). Manipulation of the n-3 polyunsaturated fatty acid content of muscle and adipose tissue in lambs1. *Journal of Animal Science*, *82*(5), 1461–1470. https://doi.org/10.2527/2004.8251461x

Corah, L. R., Dunn, T. G., & Kaltenbach, C. C. (1975). Influence of Prepartum Nutrition on the Reproductive Performance of Beef Females and the Performance of Their Progeny. *Journal of Animal Science*, *41*(3), 819–824. https://doi.org/10.2527/jas1975.413819x

De Barbieri, I., Hegarty, R. S., Silveira, C., Gulino, L. M., Oddy, V. H., Gilbert, R. A., Klieve, A. V, & Ouwerkerk, D. (2015). Programming rumen bacterial communities in newborn Merino lambs. *Small Ruminant Research*, *129*, 48–59. https://doi.org/https://doi.org/10.1016/j.smallrumres.2015.05.015

Demeyer, D., & Doreau, M. (1999). Targets and procedures for altering ruminant meat and milk lipids. *Proceedings of the Nutrition Society*, *58*(3), 593–607. https://doi.org/DOI: 10.1017/S0029665199000786

Dempsey, E. W. (1937). Follicular growth rate and ovulation after various experimental procedures in the guinea pig. *American Journal of Physiology-Legacy Content*, *120*(1), 126–132.

DeRouen, S. M., Franke, D. E., Morrison, D. G., Wyatt, W. E., Coombs, D. F., White, T. W., Humes, P. E., & Greene, B. B. (1994). Prepartum body condition and weight influences on reproductive performance of first-calf beef cows. *Journal of Animal Science*, *72*(5), 1119–1125. https://doi.org/10.2527/1994.7251119x

Díaz, M. T., Pérez, C., Sánchez, C. I., Lauzurica, S., Cañeque, V., González, C., & De La Fuente, J. (2017). Feeding microalgae increases omega 3 fatty acids of fat deposits and muscles in light lambs. *Journal of Food Composition and Analysis*, *56*, 115–123. https://doi.org/https://doi.org/10.1016/j.jfca.2016.12.009

Diskin, M. G., Parr, M. H., & Morris, D. G. (2011). Embryo death in cattle: an update. *Reproduction, Fertility and Development*, *24*(1), 244–251. https://doi.org/10.1071/RD11914

Driancourt, M. A. (1991). Follicular dynamics in sheep and cattle. *Theriogenology*, *35*(1), 55–79. https://doi.org/https://doi.org/10.1016/0093-691X(91)90148-7

Driancourt, M. A. (2001). Regulation of ovarian follicular dynamics in farm animals. Implications for manipulation of reproduction. *Theriogenology*, *55*(6), 1211–1239. https://doi.org/https://doi.org/10.1016/S0093-691X(01)00479-4

Dziuk, P. J., & Bellows, R. A. (1983). Management of Reproduction of Beef Cattle, Sheep and Pigs. *Journal of Animal Science*, *57*(suppl_2), 355–379. https://doi.org/10.2527/animalsci1983.57Supplement_2355x

El Hassan Belarbi, E. M., & Chisti, Y. (2000). A process for high yield and scaleable recovery of high purity eicosapentaenoic acid esters from microalgae and fish oil. *Enzyme and Microbial Technology*, *26*, 516–529.

Endo, H., Nakajima, K., Chino, R., & Shirota, M. (1974). Growth Characteristics and Cellular Components of Chlorella regularis, Heterotrophic Fast Growing Strain. *Agricultural and Biological Chemistry*, *38*(1), 9–18. https://doi.org/10.1080/00021369.1974.10861121

Erickson, B. H. (1966). Development and Senescence of the Postnatal Bovine Ovary. *Journal of Animal Science*, *25*(3), 800–805. https://doi.org/10.2527/jas1966.253800x

Fair, T. (2003). Follicular oocyte growth and acquisition of developmental competence. *Animal Reproduction Science*, *78*(3), 203–216. https://doi.org/https://doi.org/10.1016/S0378-4320(03)00091-5

Ferguson, J. D., Sklan, D., Chalupa, W. V, & Kronfeld, D. S. (1990). Effects of Hard Fats on In Vitro and In Vivo Rumen Fermentation, Milk Production, and Reproduction in Dairy Cows1. *Journal of Dairy Science*, *73*(10), 2864–2879. https://doi.org/https://doi.org/10.3168/jds.S0022-0302(90)78974-6

Ferrell, C. L. (1989). *Placental Regulation of Fetal Growth BT - Animal Growth Regulation* (D. R. Campion, G. J. Hausman, & R. J. Martin (eds.); pp. 1–19). Springer US. https://doi.org/10.1007/978-1-4684-8872-2_1

Ford, S. P. (1995). Control of blood flow to the gravid uterus of domestic livestock species. *Journal of Animal Science*, *73*(6), 1852–1860. https://doi.org/10.2527/1995.7361852x

Fortune, J. E. (1994). Ovarian Follicular Growth and Development in Mammals1. *Biology of Reproduction*, *50*(2), 225–232. https://doi.org/10.1095/biolreprod50.2.225

Fouladi-Nashta, A. A., Gutierrez, C. G., Gong, J. G., Garnsworthy, P. C., & Webb, R. (2007). Impact of Dietary Fatty Acids on Oocyte Quality and Development in Lactating Dairy Cows1. *Biology of Reproduction*, *77*(1), 9–17. https://doi.org/10.1095/biolreprod.106.058578

Franklin, S. T., Martin, K. R., Baer, R. J., Schingoethe, D. J., & Hippen, A. R. (1999). Dietary Marine Algae (Schizochytrium sp.) Increases Concentrations of Conjugated Linoleic, Docosahexaenoic and Transvaccenic Acids in Milk of Dairy Cows. *The Journal of Nutrition*, *129*(11), 2048–2054. https://doi.org/10.1093/jn/129.11.2048

Gibbons, J. R., Wiltbank, M. C., & Ginther, O. J. (1997). Functional Interrelationships between Follicles Greater than 4 mm and the Follicle-Stimulating Hormone Surge in Heifers1. *Biology of Reproduction*, *57*(5), 1066–1073. https://doi.org/10.1095/biolreprod57.5.1066

Ginther, O. J., Wiltbank, M. C., Fricke, P. M., Gibbons, J. R., & Kot, K. (1996). Selection of the dominant follicle in cattle. *Biology of Reproduction*, *55*(6), 1187–1194.

Glover, K. E., Budge, S., Rose, M., Rupasinghe, H. P. V, MacLaren, L., Green-Johnson, J., & Fredeen, A. H. (2012). Effect of feeding fresh forage and marine algae on the fatty acid composition and oxidation of milk and butter. *Journal of Dairy Science*, *95*(6), 2797–2809. https://doi.org/https://doi.org/10.3168/jds.2011-4736

Grazul-Bilska, A. T., Caton, J. S., Arndt, W., Burchill, K., Thorson, C., Borowczyk, E., Bilski, J. J., Redmer, D. A., Reynolds, L. P., & Vonnahme, K. A. (2009). Cellular proliferation and vascularization in ovine fetal ovaries: effects of undernutrition and selenium in maternal diet. *Reproduction*, *137*(4), 699.

Gulati, S. K., Scott, T. W., & Ashes, J. R. (1997). In-vitro assessment of fat supplements for ruminants. *Animal Feed Science and Technology*, *64*(2), 127–132. https://doi.org/https://doi.org/10.1016/S0377-8401(96)01063-2

Gunn, P. J., Schoonmaker, J. P., Lemenager, R. P., & Bridges, G. A. (2014). Feeding excess crude protein to gestating and lactating beef heifers: Impact on parturition, milk composition, ovarian function, reproductive efficiency and pre-weaning progeny growth. *Livestock Science*, *167*, 435–448.

Hansel, W., Concannon, P. W., & Lukaszewska, J. H. (1973). Corpora Lutea of the Large Domestic Animals. *Biology of Reproduction*, *8*(2), 222–245. https://doi.org/10.1093/biolreprod/8.2.222

Hansel, W., & Convey, E. M. (1983). Physiology of the Estrous Cycle1. *Journal of Animal Science*, *57*(suppl_2), 404–424. https://doi.org/10.2527/animalsci1983.57Supplement_2404x

Harel, M., Koven, W., Lein, I., Bar, Y., Behrens, P., Stubblefield, J., Zohar, Y., & Place, A. R. (2002). Advanced DHA, EPA and ArA enrichment materials for marine aquaculture using single cell heterotrophs. *Aquaculture*, *213*(1), 347–362. https://doi.org/https://doi.org/10.1016/S0044-8486(02)00047-9

Henderson, K. M., & Franchimont, P. (1981). Regulation of inhibin production by bovine ovarian cells in vitro. *Reproduction*, *63*(2), 431–442. https://doi.org/10.1530/jrf.0.0630431

Herd, D. B., & Sprott, L. R. (1986). Body condition, nutrition and reproduction of beef cows. *Texas FARMER Collection*.

Herdt, T. H. (2000). Ruminant Adaptation to Negative Energy Balance: Influences on the Etiology of Ketosis and Fatty Liver. *Veterinary Clinics: Food Animal Practice*, *16*(2), 215–230. https://doi.org/10.1016/S0749-0720(15)30102-X

Hersom, M. (2007). Basic Nutrient Requirements of Beef Cows. *EDIS*, *2007*(20 SE-Historical Fact Sheets). https://journals.flvc.org/edis/article/view/117029

Hess, B. W., Moss, G. E., & Rule, D. C. (2008). A decade of developments in the area of fat supplementation research with beef cattle and sheep1. *Journal of Animal Science*, *86*(suppl_14), E188–E204. https://doi.org/10.2527/jas.2007-0546

Hightshoe, R. B., Cochran, R. C., Corah, L. R., Kiracofe, G. H., Harmon, D. L., & Perry, R. C. (1991). Effects of calcium soaps of fatty acids on postpartum reproductive function in beef cows. *Journal of Animal Science*, *69*(10), 4097–4103. https://doi.org/10.2527/1991.69104097x

Hixon, J. E., & Hansel, W. (1974). Evidence for Preferential Transfer of Prostaglandin F2α to the Ovarian Artery Following Intrauterine Administration in Cattle1. *Biology of Reproduction*, *11*(5), 543–552. https://doi.org/10.1095/biolreprod11.5.543

Homa, S. T., & Brown, C. A. (1992). Changes in linoleic acid during follicular development and inhibition of spontaneous breakdown of germinal vesicles in cumulus-free bovine oocytes. *Reproduction*, *94*(1), 153–160. https://doi.org/10.1530/jrf.0.0940153

Hopkins, D. L., Clayton, E. H., Lamb, T. A., van de Ven, R. J., Refshauge, G., Kerr, M. J., Bailes, K., Lewandowski, P., & Ponnampalam, E. N. (2014). The impact of supplementing lambs with algae on growth, meat traits and oxidative status. *Meat Science*, *98*(2), 135–141. https://doi.org/https://doi.org/10.1016/j.meatsci.2014.05.016

Hostens, M., Fievez, V., Vlaeminck, B., Buyse, J., Leroy, J., Piepers, S., De Vliegher, S., & Opsomer, G. (2011). The effect of marine algae in the ration of high-yielding dairy cows during transition on metabolic parameters in serum and follicular fluid around parturition. *Journal of Dairy Science*, *94*(9), 4603–4615. https://doi.org/https://doi.org/10.3168/jds.2010-3899

Hubbert, W. T., OHV, S., & GD, B. (1972). *Changes in organ weights and fluid volumes during growth of the bovine fetus*.

Inskeep, E. K. (1973). Potential Uses of Prostaglandins in Control of Reproductive Cycles of Domestic Animals. *Journal of Animal Science*, *36*(6), 1149–1157. https://doi.org/10.2527/jas1973.3661149x

IRELAND, J. J., & ROCHE, J. F. (1983). Development of Nonovulatory Antral Follicles in Heifers: Changes in Steroids in Follicular Fluid and Receptors for Gonadotr opins*. *Endocrinology*, *112*(1), 150–156. https://doi.org/10.1210/endo-112-1-150

Jacobs, R. D., Ealy, A. D., Pennington, P. M., Pukazhenthi, B., Warren, L. K., Wagner, A. L., Johnson, A. K., Hess, T. M., Knight, J. W., & Splan, R. K. (2018). Dietary Supplementation of Algae-Derived Omega-3 Fatty Acids Influences Endometrial and Conceptus Transcript Profiles in Mares. *Journal of Equine Veterinary Science*, *62*, 66–75. https://doi.org/https://doi.org/10.1016/j.jevs.2017.08.001

JG, A. D. G. G., & Webb, R. (2003). Interactions between nutrition and ovarian activity in cattle: physiological cellular and molecular mechanisms. *Reproduction Supplement*, *61*, 403–414.

Kapalka, G. M. (2009). *Nutritional and herbal therapies for children and adolescents: a handbook for mental health clinicians*. Academic Press.

Kinder, J. E., Kojima, F. N., Bergfeld, E. G. M., Wehrman, M. E., & Fike, K. E. (1996). Progestin and estrogen regulation of pulsatile LH release and development of persistent ovarian follicles in cattle. *Journal of Animal Science*, *74*(6), 1424–1440. https://doi.org/10.2527/1996.7461424x

Kojima, N., Stumpf, T. T., Cupp, A. S., Werth, L. A., Roberson, M. S., Wolfe, M. W., Kittok, R. J., & Kinder, J. E. (1992). Exogenous Progesterone and Progestins as Used in Estrous Synchrony Regimens do not Mimic the Corpus Luteum in Regulation of Luteinizing Hormone and 17β-Estradiol in Circulation of Cows1. *Biology of Reproduction*, *47*(6), 1009–1017. https://doi.org/10.1095/biolreprod47.6.1009

Lammoglia, M. A., Willard, S. T., Hallford, D. M., & Randel, R. D. (1997). Effects of dietary fat on follicular development and circulating concentrations of lipids, insulin, progesterone, estradiol-17β, 13,14-dihydro-15-keto-prostaglandin F2α, and growth hormone in estrous cyclic Brahman cows. *Journal of Animal Science*, *75*(6), 1591–1600. https://doi.org/10.2527/1997.7561591x

Lents, C. A., Peel, R. K., Seidel Jr, G. E., & Niswender, G. D. (2003). *Reproduction on the ranch*.

Levine, L., & Worth, N. (1984). Eicosapentaenoic acid: Its effects on arachidonic acid metabolism by cells in culture. *Journal of Allergy and Clinical Immunology*, *74*(3, Part 2), 430–436. https://doi.org/https://doi.org/10.1016/0091-6749(84)90143-X

Lippmeier, J. C., Crawford, K. S., Owen, C. B., Rivas, A. A., Metz, J. G., & Apt, K. E. (2009). Characterization of Both Polyunsaturated Fatty Acid Biosynthetic Pathways in Schizochytrium sp. *Lipids*, *44*(7), 621–630. https://doi.org/10.1007/s11745-009-3311-9

Lopes, C. N., Scarpa, A. B., Cappellozza, B. I., Cooke, R. F., & Vasconcelos, J. L. M. (2009). Effects of rumen-protected polyunsaturated fatty acid supplementation on reproductive performance of Bos indicus beef cows1. *Journal of Animal Science*, *87*(12), 3935–3943. https://doi.org/10.2527/jas.2009-2201

Lucy, M. C., Staples, C. R., Michel, F. M., & Thatcher, W. W. (1991). Energy Balance and Size and Number of Ovarian Follicles Detected by Ultrasonography in Early Postpartum Dairy Cows1. *Journal of Dairy Science*, *74*(2), 473–482. https://doi.org/https://doi.org/10.3168/jds.S0022-0302(91)78194-0

Lucy, M. C., Staples, C. R., Thatcher, W. W., Erickson, P. S., Cleale, R. M., Firkins, J. L., Clark, J. H., Murphy, M. R., & Brodie, B. O. (1992). Influence of diet composition, dry-matter

intake, milk production and energy balance on time of post-partum ovulation and fertility in dairy cows. *Animal Science*, *54*(3), 323–331. https://doi.org/DOI: 10.1017/S0003356100020778

Lum, K. K., Kim, J., & Lei, X. G. (2013). Dual potential of microalgae as a sustainable biofuel feedstock and animal feed. *Journal of Animal Science and Biotechnology*, *4*(1), 53. https://doi.org/10.1186/2049-1891-4-53

Lussier-Cacan, S., Bolte, E., Bidallier, M., Huang, Y. S., & Davignon, J. (1977). Cyclic Fluctuations of Plasma Cholesterol in the Female Miniature Swine and Its Relationship to Progesterone Secretion. *Proceedings of the Society for Experimental Biology and Medicine*, *154*(3), 471–474. https://doi.org/10.3181/00379727-154-39696a

Madeira, M. S., Cardoso, C., Lopes, P. A., Coelho, D., Afonso, C., Bandarra, N. M., & Prates, J. A. M. (2017). Microalgae as feed ingredients for livestock production and meat quality: A review. *Livestock Science*, *205*, 111–121. https://doi.org/https://doi.org/10.1016/j.livsci.2017.09.020

Marei, W. F., Wathes, D. C., & Fouladi-Nashta, A. A. (2010). Impact of linoleic acid on bovine oocyte maturation and embryo development. *Reproduction*, *139*(6), 979.

Marques, J. A., Del Valle, T. A., Ghizzi, L. G., Zilio, E. M. C., Gheller, L. S., Nunes, A. T., Silva, T. B. P., Dias, M. S. da S., Grigoletto, N. T. S., & Koontz, A. F. (2019). Increasing dietary levels of docosahexaenoic acid-rich microalgae: Ruminal fermentation, animal performance, and milk fatty acid profile of mid-lactating dairy cows. *Journal of Dairy Science*, *102*(6), 5054–5065.

Marsh, J. M., & Savard, K. (1966). The stimulation of progesterone synthesis in bovine corpora lutea by adenosine 3', 5'-monophosphate (1). *Steroids*, *8*(2), 133–148. https://doi.org/https://doi.org/10.1016/0039-128X(66)90088-2

Matsuo, H., Baba, Y., Nair, R. M. G., Arimura, A., & Schally, A. V. (1971). Structure of the porcine LH- and FSH-releasing hormone. I. The proposed amino acid sequence. *Biochemical and Biophysical Research Communications*, *43*(6), 1334–1339. https://doi.org/https://doi.org/10.1016/S0006-291X(71)80019-0

Matton, P., Adelakoun, V., Couture, Y., & Dufour, J. J. (1981). Growth and Replacement of the Bovine Ovarian Follicles during the Estrous Cycle1. *Journal of Animal Science*, *52*(4), 813–820. https://doi.org/10.2527/jas1981.524813x

Mattos, R., Staples, C. R., & Thatcher, W. W. (2000). Effects of dietary fatty acids on reproduction in ruminants. *Reviews of Reproduction*, *5*, 38–45.

Meale, S. J., Chaves, A. V, He, M. L., & McAllister, T. A. (2014). Dose–response of supplementing marine algae (Schizochytrium spp.) on production performance, fatty acid

profiles, and wool parameters of growing lambs1. *Journal of Animal Science*, *92*(5), 2202–2213. https://doi.org/10.2527/jas.2013-7024

Metcalfe, J., Stock, M. K., & Barron, D. H. (1988). Maternal physiology during gestation. The Physiology of Reproduction. *Knobil E., Neill J*.

Metherel, A. H., Irfan, M., Klingel, S. L., Mutch, D. M., & Bazinet, R. P. (2019). Compound-specific isotope analysis reveals no retroconversion of DHA to EPA but substantial conversion of EPA to DHA following supplementation: a randomized control trial. *The American Journal of Clinical Nutrition*, *110*(4), 823–831.

Mihm, M., & Bleach, E. C. L. (2003). Endocrine regulation of ovarian antral follicle development in cattle. *Animal Reproduction Science*, *78*(3), 217–237. https://doi.org/https://doi.org/10.1016/S0378-4320(03)00092-7

Moran, C. A., Morlacchini, M., Keegan, J. D., & Fusconi, G. (2018). The effect of dietary supplementation with Aurantiochytrium limacinum on lactating dairy cows in terms of animal health, productivity and milk composition. *Journal of Animal Physiology and Animal Nutrition*, *102*(2), 576–590.

Moran, L. A., Scrimgeour, K. G., Horton, H. R., Ochs, R. S., & Rawn, J. D. (1994). *Biochemistry, ; MORAN, LA; ed*. Neil Patterson Publisher–Prentice Hall: Englewood Cliffs NJ;

Ndimba, B. K., Ndimba, R. J., Johnson, T. S., Waditee-Sirisattha, R., Baba, M., Sirisattha, S., Shiraiwa, Y., Agrawal, G. K., & Rakwal, R. (2013). Biofuels as a sustainable energy source: An update of the applications of proteomics in bioenergy crops and algae. *Journal of Proteomics*, *93*, 234–244. https://doi.org/https://doi.org/10.1016/j.jprot.2013.05.041

Nikoloff, N., Campagna, A., Luchetti, C., Carranza-Martín, A. C., Pascua, A. M., Anchordoquy, J. M., Anchordoquy, J. P., Lombardo, D. M., Seoane, A., & Furnus, C. C. (2019). Effects of EPA on bovine oocytes matured in vitro with antioxidants: Impact on the lipid content of oocytes and early embryo development. *Theriogenology*. https://doi.org/https://doi.org/10.1016/j.theriogenology.2019.11.028

Niswender, G. D., Reimers, T. J., Diekman, M. A., & Nett, T. M. (1976). Blood Flow: A Mediator of Ovarian Function1. *Biology of Reproduction*, *14*(1), 64–81. https://doi.org/10.1095/biolreprod14.1.64

Nute, G. R., Richardson, R. I., Wood, J. D., Hughes, S. I., Wilkinson, R. G., Cooper, S. L., & Sinclair, L. A. (2007). Effect of dietary oil source on the flavour and the colour and lipid stability of lamb meat. *Meat Science*, *77*(4), 547–555. https://doi.org/https://doi.org/10.1016/j.meatsci.2007.05.003

Oldick, B. S., Staples, C. R., Thatcher, W. W., & Gyawu, P. (1997). Abomasal Infusion of Glucose and Fat—Effect on Digestion, Production, and Ovarian and Uterine Functions of

Cows1. *Journal of Dairy Science*, *80*(7), 1315–1328. https://doi.org/https://doi.org/10.3168/jds.S0022-0302(97)76060-0

Palmquist, D. L., & Moser, E. A. (1981). Dietary fat effects on blood insulin, glucose utilization, and milk protein content of lactating cows. *Journal of Dairy Science*, *64*(8), 1664–1670.

Pate, J. L., Johnson-Larson, C. J., & Ottobre, J. S. (2012). Life or death decisions in the corpus luteum. *Reproduction in Domestic Animals*, *47*, 297–303.

Patterson, D. J., Perry, R. C., Kiracofe, G. H., Bellows, R. A., Staigmiller, R. B., & Corah, L. R. (1992). Management considerations in heifer development and puberty. *Journal of Animal Science*, *70*(12), 4018–4035. https://doi.org/10.2527/1992.70124018x

Petit, H. V, & Twagiramungu, H. (2006). Conception rate and reproductive function of dairy cows fed different fat sources. *Theriogenology*, *66*(5), 1316–1324. https://doi.org/https://doi.org/10.1016/j.theriogenology.2006.04.029

Petit, H. V, Gagnon, N., & Palin, M.-F. (2009). Influence of Fatty Acids on Reproduction and Immune Function in Ruminants. *30 Th Western Nutrition Conference*, 139.

Popp, J., Harangi-Rákos, M., Gabnai, Z., Balogh, P., Antal, G., & Bai, A. (2016). Biofuels and their co-products as livestock feed: global economic and environmental implications. *Molecules*, *21*(3), 285.

Posser, C. J. M., Almeida, L. M., Moreira, F., Bianchi, I., Gasperin, B. G., & Lucia Jr, T. (2018). Supplementation of diets with omega-3 fatty acids from microalgae: Effects on sow reproductive performance and metabolic parameters. *Livestock Science*, *207*, 59–62.

Priyadarshani, I., & Rath, B. (2012). Commercial and industrial applications of micro algae–A review. *Journal of Algal Biomass Utilization*, *3*(4), 89–100.

Ratledge, C. (2013). Microbial oils: an introductory overview of current status and future prospects. *Ocl*, *20*(6), D602.

Reynolds, L. P., & Redmer, D. A. (2001). Angiogenesis in the Placenta1. *Biology of Reproduction*, *64*(4), 1033–1040. https://doi.org/10.1095/biolreprod64.4.1033

Reynolds, L. P., & Redmer, D. A. (1995). Utero-placental vascular development and placental function. *Journal of Animal Science*, *73*(6), 1839–1851. https://doi.org/10.2527/1995.7361839x

Ribeiro, T., Lordelo, M. M., Alves, S. P., Bessa, R. J. B., Costa, P., Lemos, J. P. C., Ferreira, L. M. A., Fontes, C. M. G. A., & Prates, J. A. M. (2013). Direct supplementation of diet is the most efficient way of enriching broiler meat with n-3 long-chain polyunsaturated fatty acids. *British Poultry Science*, *54*(6), 753–765. https://doi.org/10.1080/00071668.2013.841861

Ribeiro, T., Lordelo, M. M., Costa, P., Alves, S. P., Benevides, W. S., Bessa, R. J. B., Lemos, J. P. C., Pinto, R. M. A., Ferreira, L. M. A., Fontes, C. M. G. A., & Prates, J. A. M. (2014). Effect of reduced dietary protein and supplementation with a docosahexaenoic acid product on broiler performance and meat quality. *British Poultry Science*, *55*(6), 752–765. https://doi.org/10.1080/00071668.2014.971222

Ricciotti, E., & FitzGerald, G. A. (2011). Prostaglandins and inflammation. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *31*(5), 986–1000.

Richmond, A. (2004). Biological principles of mass cultivation. *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*, 125–177.

Roberts, R. M., Leaman, D. W., & Cross, J. C. (1992). Role of Interferons in Maternal Recognition of Pregnancy in Ruminants. *Proceedings of the Society for Experimental Biology and Medicine*, *200*(1), 7–18. https://doi.org/10.3181/00379727-200-43387A

Robinson, J. J., & McDonald, I. (1979). Ovine prenatal growth, its mathematical description and the effects of maternal nutrition. *Annales de Biologie Animale Biochimie Biophysique*, *19*(1B), 225–234.

Robinson, R. S., Pushpakumara, P. G. A., Cheng, Z., Peters, A. R., Abayasekara, D. R. E., & Wathes, D. C. (2002). Effects of dietary polyunsaturated fatty acids on ovarian and uterine function in lactating dairy cows. *REPRODUCTION-CAMBRIDGE-*, *124*(1), 119–131.

Roche, J. F., Mihm, M., Diskin, M. G., & Ireland, J. J. (1998). A Review of Regulation of Follicle Growth in Cattle. *Journal of Animal Science*, *76*(suppl_3), 16–29. https://doi.org/10.2527/1998.76suppl_316x

Roselli, C. F. (2007). Brain aromatase: Roles in reproduction and neuroprotection. *The Journal of Steroid Biochemistry and Molecular Biology*, *106*(1), 143–150. https://doi.org/https://doi.org/10.1016/j.jsbmb.2007.05.014

Ryan, D. P., Bao, B., Griffith, M. K., & Williams, G. L. (1995). Metabolic and luteal sequelae to heightened dietary fat intake in undernourished, anestrous beef cows induced to ovulate. *Journal of Animal Science*, *73*(7), 2086–2093. https://doi.org/10.2527/1995.7372086x

Salisbury, G. W., VanDemark, N. L., & Lodge, J. R. (1978). *Physiology of reproduction and artificial insemination of cattle.* (Issue Ed. 2). W.H. Freeman and Company.

Sampels, S., Turner, T., Öström, Å., & Pickova, J. (2010). Effects of α-linolenic acid and eicosapentaenoic acid from linseed and algae, respectively, on reindeer (Rangifer tarandus tarandus L.) muscle fatty acid composition. *Acta Agriculturae Scandinavica, Section A — Animal Science*, *60*(3), 175–186. https://doi.org/10.1080/09064702.2010.523092

Santos, J. E. P., Bilby, T. R., Thatcher, W. W., Staples, C. R., & Silvestre, F. T. (2008). Long chain fatty acids of diet as factors influencing reproduction in cattle. *Reproduction in Domestic Animals*, *43*, 23–30.

Sardi, L., Martelli, G., Lambertini, L., Parisini, P., & Mordenti, A. (2006). Effects of a dietary supplement of DHA-rich marine algae on Italian heavy pig production parameters. *Livestock Science*, *103*(1), 95–103. https://doi.org/https://doi.org/10.1016/j.livsci.2006.01.009

Savio, J. D., Keenan, L., Boland, M. P., & Roche, J. F. (1988). Pattern of growth of dominant follicles during the oestrous cycle of heifers. *Reproduction*, *83*(2), 663–671. https://doi.org/10.1530/jrf.0.0830663

Schallenberger, E., Schams, D., Bullermann, B., & Walters, D. L. (1984). Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during prostaglandin-induced regression of the corpus luteum in the cow. *Reproduction*, *71*(2), 493–501. https://doi.org/10.1530/jrf.0.0710493

Schams, D., Schallenberger, E., Hoffmann, B., & Karg, H. (1977). The oestrous cycle of the cow: hormonal parameters and time relationships concerning oestrus, ovulation, and electrical resistance of the vaginal mucus. *European Journal of Endocrinology*, *86*(1), 180–192.

Senger, P. L. (2012). *Pathways to pregnancy & parturition*.

Shee, C. N., Lemenager, R. P., & Schoonmaker, J. P. (2018). Feeding dried distillers grains with solubles to lactating beef cows: impact of excess protein and fat on post-weaning progeny growth, glucose tolerance and carcass traits. *Animal*, *12*(4), 750–756. https://doi.org/DOI: 10.1017/S1751731117002099

Shingfield, K. J., Kairenius, P., Ärölä, A., Paillard, D., Muetzel, S., Ahvenjärvi, S., Vanhatalo, A., Huhtanen, P., Toivonen, V., Griinari, J. M., & Wallace, R. J. (2012). Dietary Fish Oil Supplements Modify Ruminal Biohydrogenation, Alter the Flow of Fatty Acids at the Omasum, and Induce Changes in the Ruminal Butyrivibrio Population in Lactating Cows. *The Journal of Nutrition*, *142*(8), 1437–1448. https://doi.org/10.3945/jn.112.158576

SHORT, R. E., & ADAMS, D. C. (1988). NUTRITIONAL AND HORMONAL INTERRELATIONSHIPS IN BEEF CATTLE REPRODUCTION. *Canadian Journal of Animal Science*, *68*(1), 29–39. https://doi.org/10.4141/cjas88-003

Silvestre, F. T., Carvalho, T. S. M., Crawford, P. C., Santos, J. E. P., Staples, C. R., Jenkins, T., & Thatcher, W. W. (2011). Effects of differential supplementation of fatty acids during the peripartum and breeding periods of Holstein cows: II. Neutrophil fatty acids and function, and acute phase proteins. *Journal of Dairy Science*, *94*(5), 2285–2301.

Sinedino, L. D. P., Honda, P. M., Souza, L. R. L., Lock, A. L., Boland, M. P., Staples, C. R., Thatcher, W. W., & Santos, J. E. P. (2017). Effects of supplementation with docosahexaenoic acid on reproduction of dairy cows. *Reproduction*, *153*(5), 707–723.

Sklan, D., Kaim, M., Moallem, U., & Folman, Y. (1994). Effect of Dietary Calcium Soaps on Milk Yield, Body Weight, Reproductive Hormones, and Fertility in First Parity and Older Cows. *Journal of Dairy Science*, *77*(6), 1652–1660. https://doi.org/10.3168/jds.S0022-0302(94)77107-1

SMEATON, T. C., & ROBERTSON, H. A. (1971). STUDIES ON THE GROWTH AND ATRESIA OF GRAAFIAN FOLLICLES IN THE OVARY OF THE SHEEP. *Reproduction*, *25*(2), 243–252. https://doi.org/10.1530/jrf.0.0250243

Sousa, I., Gouveia, L., Batista, A. P., Raymundo, A., & Bandarra, N. M. (2008). Microalgae in novel food products. *Food Chemistry Research Developments*, 75–112.

Spencer, T. E., & Bazer, F. W. (2002). Biology of progesterone action during pregnancy recognition and maintenance of pregnancy. *Front Biosci*, *7*(7), d1879–d1898.

Spicer, L. J., Alpizar, E., & Echternkamp, S. E. (1993). Effects of insulin, insulin-like growth factor I, and gonadotropins on bovine granulosa cell proliferation, progesterone production, estradiol production, and(or) insulin-like growth factor I production in vitro1. *Journal of Animal Science*, *71*(5), 1232–1241. https://doi.org/10.2527/1993.7151232x

Spicer, L. J., & Echternkamp, S. E. (1995). The ovarian insulin and insulin-like growth factor system with an emphasis on domestic animals. *Domestic Animal Endocrinology*, *12*(3), 223–245. https://doi.org/https://doi.org/10.1016/0739-7240(95)00021-6

Spolaore, P., Joannis-Cassan, C., Duran, E., & Isambert, A. (2006). Commercial applications of microalgae. *Journal of Bioscience and Bioengineering*, *101*(2), 87–96. https://doi.org/https://doi.org/10.1263/jbb.101.87

Stamey, J. A., Shepherd, D. M., de Veth, M. J., & Corl, B. A. (2012). Use of algae or algal oil rich in n-3 fatty acids as a feed supplement for dairy cattle. *Journal of Dairy Science*, *95*(9), 5269–5275. https://doi.org/https://doi.org/10.3168/jds.2012-5412

Staples, C. R., Burke, J. M., & Thatcher, W. W. (1998). Influence of Supplemental Fats on Reproductive Tissues and Performance of Lactating Cows1. *Journal of Dairy Science*, *81*(3), 856–871. https://doi.org/https://doi.org/10.3168/jds.S0022-0302(98)75644-9

Staples, C. R., Amaral, B. C., & Thatcher, W. W. (n.d.). *Fat and Fat-Soluble Vitamin Supplementation for Improving Reproduction of the Dairy Cow*.

Stumpf, T. T., Day, M. L., Wolfe, M. W., Clutter, A. C., Stotts, J. A., Wolfe, P. L., Kittok, R. J., & Kinder, J. E. (1989). Effect of Estradiol on Secretion of Luteinizing Hormone during the

Follicular Phase of the Bovine Estrous Cycle1. *Biology of Reproduction*, *41*(1), 91–97. https://doi.org/10.1095/biolreprod41.1.91

Szczechowiak, J., Szumacher-Strabel, M., El-Sherbiny, M., Pers-Kamczyc, E., Pawlak, P., & Cieslak, A. (2016). Rumen fermentation, methane concentration and fatty acid proportion in the rumen and milk of dairy cows fed condensed tannin and/or fish-soybean oils blend. *Animal Feed Science and Technology*, *216*, 93–107. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2016.03.014

Taylor, V. J., Cheng, Z., Pushpakumara, P. G. A., Wathes, D. C., & Beever, D. E. (2004). Relationships between the plasma concentrations of insulin-like growth factor-I in dairy cows and their fertility and milk yield. *Veterinary Record*, *155*(19), 583 LP – 588. https://doi.org/10.1136/vr.155.19.583

Thatcher, W. W., Meyer, M. D., & Danet-Desnoyers, G. (1995). Maternal recognition of pregnancy. *Journal of Reproduction and Fertility-Supplements Only*, *49*, 15–28.

Thomas, M. G., Bao, B., & Williams, G. L. (1997). Dietary fats varying in their fatty acid composition differentially influence follicular growth in cows fed isoenergetic diets. *Journal of Animal Science*, *75*(9), 2512–2519. https://doi.org/10.2527/1997.7592512x

Till, B. E., Huntington, J. A., Kliem, K. E., Taylor-Pickard, J., & Sinclair, L. A. (2020). Long term dietary supplementation with microalgae increases plasma docosahexaenoic acid in milk and plasma but does not affect plasma 13,14-dihydro-15-keto PGF2α concentration in dairy cows. *Journal of Dairy Research*, *87*(1), 14–22. https://doi.org/DOI: 10.1017/S002202991900102X

Toral, P. G., Belenguer, A., Shingfield, K. J., Hervás, G., Toivonen, V., & Frutos, P. (2012). Fatty acid composition and bacterial community changes in the rumen fluid of lactating sheep fed sunflower oil plus incremental levels of marine algae. *Journal of Dairy Science*, *95*(2), 794–806. https://doi.org/https://doi.org/10.3168/jds.2011-4561

Vahmani, P., Fredeen, A. H., & Glover, K. E. (2013). Effect of supplementation with fish oil or microalgae on fatty acid composition of milk from cows managed in confinement or pasture systems. *Journal of Dairy Science*, *96*(10), 6660–6670. https://doi.org/https://doi.org/10.3168/jds.2013-6914

Vanbergue, E., Peyraud, J.-L., & Hurtaud, C. (2018). Effects of new n-3 fatty acid sources on milk fatty acid profile and milk fat properties in dairy cows. *Journal of Dairy Research*, *85*(3), 265–272.

Vlcek, M., Andrlikova, M., Barbato, O., Bina, V., Boland, M. P., Dolezel, R., Lopatarova, M., & Cech, S. (2017). Supplementation of dairy cows with docosahexaenoic acid did not affect ovarian activity. *Czech Journal of Animal Science*, *62*(11), 457–465.

Walters, D. L., Schams, D., & Schallenberger, E. (1984). Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during the luteal phase of the oestrous cycle in the cow. *Reproduction, 71*(2), 479–491. https://doi.org/10.1530/jrf.0.0710479

Wathes, D. C., Perks, C. M., Davis, A. J., & Denning-Kendall, P. A. (1995). Regulation of Insulin-Like Growth Factor-I and Progesterone Synthesis by Insulin and Growth Hormone in the Ovine Ovary1. *Biology of Reproduction*, *53*(4), 882–889. https://doi.org/10.1095/biolreprod53.4.882

Wathes, D. C., Abayasekara, D. R. E., & Aitken, R. J. (2007). Polyunsaturated Fatty Acids in Male and Female Reproduction1. *Biology of Reproduction*, *77*(2), 190–201. https://doi.org/10.1095/biolreprod.107.060558

Webb, R., & Campbell, B. K. (2007). Development of the dominant follicle: mechanisms of selection and maintenance of oocyte quality. *Society of Reproduction and Fertility Supplement*, *64*, 141.

Wehrman, M. E., Welsh Jr., T. H., & Williams, G. L. (1991). Diet-Induced Hyperlipidemia in Cattle Modifies the Intrafollicular Cholesterol Environment, Modulates Ovarian Follicular Dynamics, and Hastens the Onset of Postpartum Luteal Activity1. *Biology of Reproduction*, *45*(3), 514–522. https://doi.org/10.1095/biolreprod45.3.514

Wettemann, R. P., Hafs, H. D., Edgerton, L. A., & Swanson, L. V. (1972). Estradiol and Progesterone in Blood Serum during the Bovine Estrous Cycle. *Journal of Animal Science*, *34*(6), 1020–1024. https://doi.org/10.2527/jas1972.3461020x

Williams, G. L. (1989). Modulation of Luteal Activity in Postpartum Beef Cows through Changes in Dietary Lipid. *Journal of Animal Science*, *67*(3), 785–793. https://doi.org/10.2527/jas1989.673785x

Williams, G. L., & Stanko, R. L. (1999). Dietary fats as reproductive nutraceuticals in beef cattle. *Proc Am Soc Anim Sci. Www. Asas. Org/Jas/Symposia/Proceedings/0915. Pdf*.

Winwood, R. J. (2015). 6 - Algal oils: Properties and processing for use in foods and supplements. In G. B. T.-S. O. and F. in F. and N. Talbot (Ed.), *Woodhead Publishing Series in Food Science, Technology and Nutrition* (pp. 159–172). Woodhead Publishing. https://doi.org/https://doi.org/10.1016/B978-1-78242-376-8.00006-5

Wishart, D. F. (1972). Observations on the oestrous cycle of the Friesian heifer. *The Veterinary Record*, *90*(21), 595–597. https://doi.org/10.1136/vr.90.21.595

Wolfe, L. S. (1982). Eicosanoids: prostaglandins, thromboxanes, leukotrienes, and other derivatives of carbon-20 unsaturated fatty acids. *Journal of Neurochemistry*, *38*(1), 1–14.

Zachut, M., Arieli, A., Lehrer, H., Argov, N., & Moallem, U. (2008). Dietary unsaturated fatty acids influence preovulatory follicle characteristics in dairy cows. *REPRODUCTION*, *135*(5), 683–692. https://doi.org/10.1530/REP-07-0556

Zachut, M., Dekel, I., Lehrer, H., Arieli, A., Arav, A., Livshitz, L., Yakoby, S., & Moallem, U. (2010). Effects of dietary fats differing in n-6:n-3 ratio fed to high-yielding dairy cows on fatty acid composition of ovarian compartments, follicular status, and oocyte quality. *Journal of Dairy Science*, *93*(2), 529–545. https://doi.org/https://doi.org/10.3168/jds.2009-2167

Zeron, Y., Ocheretny, A., Kedar, O., Borochov, A., Sklan, D., & Arav, A. (2001). Seasonal changes in bovine fertility: relation to developmental competence of oocytes, membrane properties and fatty acid composition of follicles. *Reproduction*, *121*(3), 447–454. https://doi.org/10.1530/rep.0.1210447

Zeron, Y., Sklan, D., & Arav, A. (2002). Effect of polyunsaturated fatty acid supplementation on biophysical parameters and chilling sensitivity of ewe oocytes. *Molecular Reproduction and Development*, *61*(2), 271–278. https://doi.org/10.1002/mrd.1156

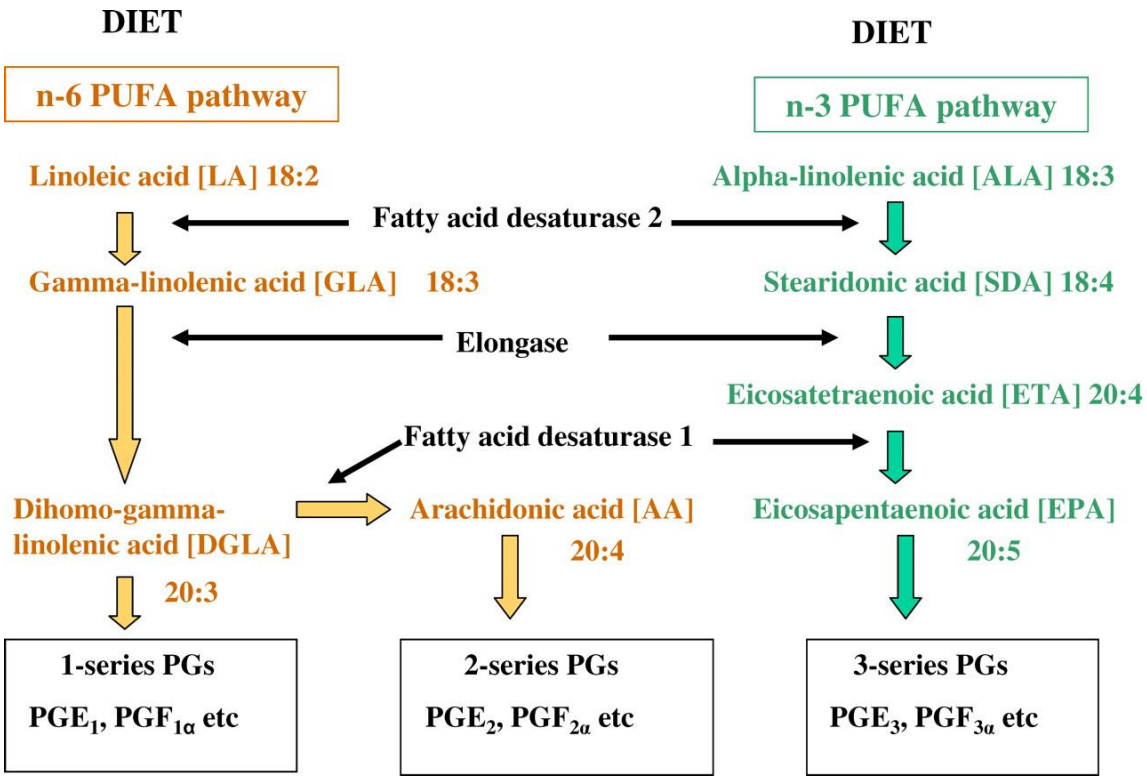

Figure 1.1. Synthesis of 1-, 2-, and 3-series prostaglandins (PG)., from Wathes et al. (2007).

CHAPTER 2. EFFECT OF SUPPLEMENTING ALGAE TO HEIFERS DURING BREEDING AND EARLY GESTATION ON GROWTH

2.1 Introduction

Fat supplementation has potential to improve reproductive performance and increase pregnancy rates in cattle (Armstrong et al., 1990, Lopes et al., 2009, Bellows et al., 2001). Fat increases energy density of the diet; however, some of the positive effects of fat seem to be influenced by the type of fatty acid fed (Santos et al., 2008). Polyunsaturated fatty acids (PUFA) play an important role in reproduction as precursors to physiologically active lipid compounds, as well as being incorporated into the phospholipids of cell membranes (Santos et al., 2013). Supplementation of omega-3 (n-3) fatty acids to dairy cows has been shown to increase uptake of n-3 fatty acids into phospholipids and mitigate immune and inflammatory responses in favor of pregnancy maintenance (Santos et al. 2008, Silvestre et al. 2011a, b). Docosohexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) are long-chain n-3 poly-unsaturated fatty acids (PUFA) that have important biological effects. Fish oil is high in EPA and DHA and has been reported to increase the concentration of phospholipids in oocyte cumulus cells and to increase follicle and oocyte numbers when supplemented to ewes (Zeron et al., 2002). Robinson et al (2002) observed significant increases in plasma estrogen, but a decrease in plasma progesterone in dairy cows supplemented with n-3 rich linseed. Ambrose et al (2006) and Zachut et al (2008) reported that n-3 supplementation increased the size of pre-ovulatory follicles and increased the number of small follicles in dairy cows. However, supplementation of n-3 fatty acids in ruminants has been associated with a decrease in circulating $PGF_{2\alpha}$ (Gulliver et al., 2012), which may delay CL regression, extend an animal's time in diestrus, and prevent ovulation (Pate et al., 2012). Series 1 and 2 prostaglandins are synthesized from n-6 PUFA and are intimately involved in uterine

involution and post-partum ovulation (Otto et al., 2014). In contrast, the 3 series prostaglandins are synthesized from n-3 PUFA and are involved in preparing the uterine environment for embryo implantation and survival. Series 3 prostaglandins improve the uterine environment by inhibiting the secretion of PGF2α, which results in increased progesterone production likely from a prolonged CL (Kim et al., 2016), increasing blastocyst cell numbers, and maintaining the pregnancy (Otto et al., 2014).

Ruminant tissues are naturally almost devoid of n-3 long-chain PUFA, specifically EPA and DHA. Fish oil is the most common ration additive used to provide very long chain n-3 fatty acids to ruminants. However, marine fish do not synthesize n-3 fatty acids; they consume microscopic algae or other algae-consuming fish to obtain n-3 fatty acids. Current technology allows for heterotrophic production of algal biomass in bulk fermenters that do not require the lighting and electricity previously needed for phototrophic algae growth (Harel et al., 2002). The production of microalgae via bulk fermentation can provide a consistent high-quality source of n-3 fatty acids (DHA and EPA) that can then be used in livestock supplementation. Increasing the content of n-3 PUFA in food products is of interest because of their role in promoting human health. Cooper et al. (2004) observed that PUFA from algae are less biohydrogenated compared to fish oil and in fact, supplemental microalgae has increased PUFA and DHA content of ruminant meat (Nute et al., 2007; Sampels et al., 2010) and milk (Stamey et al., 2012; Vahmani et al., 2013). The impact of n-3 rich microalgae supplementation on reproduction of ruminants, however, is inconsistent. Sinedino et al. (2017) observed an increase in estrous activity and cyclicity along with an increase in pregnancy rates and milk yield when feeding DHA-rich microalgae (Schizochytrium species) to postpartum dairy cows. Hostens et al. (2011) reported a similar increase in milk yield when Schizochytrium microalgae was fed to dairy cows during the

periparturient period, but observed that Schizochytrium supplementation increased the concentration of β-hydroxybutyric acid (BHBA) in follicular fluid, which in combination with a decrease in follicular glucose could be detrimental to oocyte development. The objective of the current study was to determine the effect of DHA-rich microalgae (Schizochytrium sp., DHA Gold) supplementation from 54 days prior to and 126 days after breeding on growth and reproduction of nulliparous heifers. We hypothesized that feeding microalgae to nulliparous beef heifers during the breeding season would improve reproduction without negatively effecting growth.

2.2 Materials and methods

This study was conducted in the summer and fall of 2018 (Figure 2.1) at the Purdue Animal Science Research and Education Center (ASREC) in West Lafayette, IN. Research procedures regarding the use of animals were in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010) and were approved by the Purdue Animal Care and Use Committee.

2.2.1 Reproductive tract score

Fourteen days prior to the study (d -14), reproductive tracts of the heifers were palpated, visualized using ultrasound, and scored (1-5) to determine reproductive development; where 1 = no palpable follicles, 2 = 8 mm follicles, 3 = 8-10 mm follicles, 4 = > 10 mm follicles, and 5 = > 10 mm follicles with a corpus luteum present (Andersen et al., 1991). Heifers were allotted to treatments equally based on reproductive score, BW, and breed composition; heifers with a reproductive score less than 3 were not used.

2.2.2 Animals and diets

Eighty-eight Angus x Simmental replacement heifers (427 ± 1.8 kg) were blocked by BW (heavy and light) and allotted to 2 treatments (44/treatment, 4pens/treatment, 11 heifers/pen). The two treatments consisted of no microalgae supplementation (control) or microalgae supplementation. Animals were housed in open air, mounded dry lot pens (30.5 x 48.8 m) with access to a fence-line concrete feed bunk (18.3 x 0.8 m). Control heifers were fed a diet that contained (DM basis) 52.8% mixed grass silage, 32% corn silage, and 15.2% concentrate (Table 1). Microalgae (DHA Gold, Schizochytrium species, 49% fat; 21.8% DHA; DSM Nutritionals, Inc.) was included in the microalgae diet at 1.65% of the diet DM, replacing equal parts of corn and distillers dried grains (DDGS). Diets were formulated to contain 12% CP, 0.79 Mcal/kg NEg, and meet or exceed vitamin and mineral requirements (NASEM, 2016). Diets were delivered at 0800 daily. Feed samples were collected every other week for DM determination and stored for subsequent nutrient analysis. Feed samples were oven-dried at 60˚C for 48 hours to determine DM, composited and sent to Cumberland Valley Analytical Services, Inc. (Waynesboro, PA) for nutrient analysis.

Heifers were fed treatment diets from 54 d prior to the breeding season (starting day 0 of the study) through the first trimester of gestation (180 days total). A timeline of important study dates has been provided in the figures (Figure 2.1). Initial (day 0) and final (day 180) of the treatment period BW were determined by averaging pre-prandial weights on 2 consecutive days. Heifers were also weighed prior to the AM feeding at the beginning (day 54) and end (day 98) of the breeding season to monitor BW gain. Scales (Tru-Test SR3000 scale; Tru-Test Inc., Mineral Wells, TX) weighed to the nearest 0.9 kg (< 453.6 kg) or 2.3 kg (> 453.6 kg) and were checked for accuracy at each weigh date. Body condition score (1 = emaciated, 9 = obese; Wagner et al., 1988) was assessed by the same individual at the initiation of the study, at the beginning and end

of the breeding season, and at the termination of dietary treatments. Average daily gain and DMI were determined for the pre-breeding (d 0 to 54), breeding (55 to 98), and post-breeding (99 to 180) periods as well as overall (0 to 180).

2.2.3 Reproductive measures and breeding

A subset of 24 heifers were synchronized with $PGF_{2\alpha}$ (5 ml, IM) (Lutalyse, Zoetis, Parsippany, NJ) starting on d 36 and follicular fluid was aspirated on d 47 for analysis of IGF-1 and estrogen. Follicle diameter and follicular fluid was collected from heifers that were detected in estrous (3 per pen, 12 per treatment, 24 total). Prior to follicular aspiration, heifers received an epidural of 5 ml of lidocaine (2%) to decrease peristalsis and minimize contamination during the procedure. The ovaries of the heifers were scanned with a real-time ultrasound scanner (Variable MHz linear array transducer, MicroMaxx, Sonosite, Bothell, WA) to assess the presence of follicles. The transducer was equipped with a 58-cm long holder with a needle guide. A one-way special puncture needle (68 cm long) tipped with an 8.9 cm, 17-gauge needle was pushed through the vaginal wall and inserted into the ovarian surface. The one-way needle was connected to a tubing system and syringe. All follicles greater than 5 mm were targeted for aspiration. After aspiration, follicular fluid was recovered from the tubing for sampling. The follicular fluid was placed into 1.5 mL microcentrifuge tubes, frozen, and stored at -20 ℃ until analysis. Estrogen and IGF-1 were measured by an enzyme-labeled chemiluminescent immunometric assay using the Immulite 1000 (Siemens Medical Solutions Diagnostics, Los Angeles, CA).

Heifers were synchronized with $PGF_{2\alpha}$ on d 54 of the study and artificial insemination (AI) was performed using the AM/PM rule following visual estrus detection with assistance of Estrotect (Rockway Inc., Spring Valley, WI). Heifers that were not bred during week one were administered $PGF_{2\alpha}$ on d 61 and were AI bred using the AM/PM rule. On d 67 heifers were scanned by

transrectal ultrasonography for evidence of a corpus luteum and any heifer not confirmed bred was placed on a 7-day co-synch + controlled internal drug release (CIDR) protocol. The co-synch + CIDR protocol was performed by administering a single 2 ml injection of GnRH (Cystorelin, Boehringer Ingleheim, Duluth, GA) on the same day the intravaginal progesterone inserts (Eazi-breed CIDR, Zoetis, Parsippany, NJ) were administered. The CIDRs were removed 7 days later and accompanied by an intramuscular injection of $PGF_{2\alpha}$. Timed artificial insemination along with a final injection of GnRH occurred 60-66 hours following the administration of $PGF_{2\alpha}$ on d 74. All heifers were AI bred using semen from a single sire. Pregnancy check occurred on d 82 and 109 via transrectal ultrasonography. Open heifers were removed on d 109 and placed in 1 control and 1 microalgae pen with a bull from d 123 to 141. Pregnant heifers remained in the treatment pens. There were 7-9 heifers per pen in the treatment pens after open heifers were removed. Final pregnancy rates were determined on all heifers (AI and natural service bred) on d 179 and the study ended on day 180. Post-study pregnancy rates for the following breeding season were assessed at weaning (d 550) to aid in determining if there is a prolonged effect on cow reproductive response.

2.2.4 Blood sampling and analysis

Blood was collected from a subset of 32 heifers (16 per treatment; 4 per pen) via jugular venipuncture on days 0 and 98 for analysis of fatty acid concentration and on day 180 for analysis of glucose and insulin concentrations at 0, 3, 6, and 9 hours after feeding. Heifers were chosen from pens such that BW was similar to the pen average. Blood was collected into BD vacutainer serum tubes (Becton Drive, Franklin Lakes, NJ), and temporarily stored at room temperature until centrifugation at 1250 x g for 20 minutes. The serum fraction was removed, transferred to 5 mL polystyrene tubes, and stored at -20 ℃ until analysis. Glucose concentration was determined using

a glucose oxidase/peroxidase enzyme kit (glucose liquicolor procedure No. 1070, Stanbio Laboratory, Boerne, TX) in 96 well plates. Plates were read at 495 nm in a Spark 10M plate reader (Tecan Life Sciences, Männedorf , Zürich, Switzerland). Serum was analyzed for insulin concentration using an Iodine^{125} radioimmunoassay (RIA) kit (porcine insulin RIA kit, EMD Millipore Corporation, St. Louis, MO) which was counted on a gamma counter (Wallac 1480 Wizard 3, PerkinElmer Life and Analytical Sciences, Turku, Finland).

To determine fatty acid content of feed and blood, serum was freeze dried, feed samples were oven dried, and lipid was extracted and esterified according to the procedure of Sukhija and Palmquist (1988). Samples were weighed (25 mg) into culture tubes and 2 mL of benzene containing a C13 internal standard (50 µg C13 in 1 mL of benzene) and 2.7 mL of methanol were added. The culture tubes were vortexed and 300 µL of acetyl chloride was slowly added. Tubes were then placed in a heating block for 2 hours at 70℃, cooled to room temperature, and 5 mL of 6% K_2CO_3 was added. Contents were centrifuged at 500 x g for 10 minutes and the upper organic solvent layer was transferred to a new culture tube to which 1 g of sodium sulfate was added. Then the samples were centrifuged again at 500 x g for 10 minutes and the upper organic layer was placed in 1.5 mL glass vials for analysis by gas chromatography/mass spectrometry using a Trace 1310 gas chromatograph (GC) system with Triplus RSH auto sampler coupled to a TSQ 8000 mass spectrometer (Thermo Fisher Scientific, Waltham, MA). An Agilent Select fatty acid methyl ester (FAME) column (50 m x 0.25 mm, film thickness 0.2 um) was used for the analysis (Agilent Technologies, Santa Clara, CA). The GC carrier gas was helium with a linear flow rate of 1.2 ml/min. The programmed GC temperature gradient was as follows: initial oven temperature of 75°C, increased to 175°C at a rate of 16.5°C/min. Oven temperature was held at 175°C, increased to 250°C at a rate of 5°C/min, then held at 250°C for 2 minutes. The total run time was 28 minutes.

The GC inlet temperature was set to 250°C and samples were injected in split-less mode. The MS transfer line was set to 250°C and the MS ion source was set to 250°C. All data were analyzed with Thermo Fisher Chromeleon (Version 7.2.9) software. A standard mixture of 37 FAME was purchased from Supelco (Sigma-Aldrich Corp., St. Louis, MO) and used to confirm spectra and column retention times.

2.2.5 Study conclusion and calving

Following the treatment period (d 180), heifers were managed as a single group until they weaned their first calf (d 550; 207 d post-partum). Primiparous Cow BW was taken when approximately 90% of the cows had calved (d 364). Weight of cows that had already calved on d 364 of the study was adjusted to a pregnant basis using the gravid uterine weight calculation (Ferrell, 1976) to determine pre-calving performance. Similarly, weights of cows that were still pregnant on d 364 of the study was adjusted to a non-pregnant basis using the same equation to determine post-calving performance. Cow weights and BCS were also assessed at weaning (d 550) to aid in interpretation of cow performance between the end of the study and weaning. Calf BW was assessed at birth and at weaning (d 550).

2.3 Statistical analysis

Data were analyzed as a complete randomized design with pen considered the experimental unit. Body weight, ADG, DMI, BCS, glucose, insulin, and fatty acids were analyzed as repeated measures using the MIXED procedure of SAS. The covariance structure spatial power was used. The model included the random effect of pen and the fixed effects of treatment, day, as well as the treatment x day interaction. The SLICE function of SAS was used to determine pre-planned simple

effect comparisons within time. Timed-AI and pregnancy rates were analyzed using the GLIMMIX procedure of SAS with animal as the experimental unit. Treatment comparisons were made using Fisher's protected least significant difference and the least square means (LSMEANS) statement was used to calculate adjusted means. For all variables analyzed, a P-value ≤ 0.05 was considered significant and values of $0.05 < P \leq 0.10$ were considered a tendency.

2.4 Results

2.4.1 Growth Performance

As designed, initial heifer BW (427 ± 1.8) and BCS did not differ between treatments ($P \geq 0.50$; Table 2.2). Heifer body weight did not differ on day 54 ($P = 0.80$), but on day 98, heifers supplemented with microalgae (ALG) tended ($P = 0.06$) to be heavier compared to heifers fed the control diet. At the conclusion of the study (d 180), bred ALG heifers had a greater BW ($P = 0.03$) compared to bred CON heifers. Heifers fed microalgae tended to have a greater BCS compared to control heifers on day 54 ($P = 0.10$), but BCS did not differ between treatments at any other time point during the study ($P \geq 0.63$). Daily gain (Table 2.3) was greater ($P = 0.03$) for ALG heifers during the second period (d 55 to 98), which coincided with the breeding season, but did not differ ($P \geq 0.25$) from d 0 to 54, d 99 to 180, d 0 to 180. Dry matter intake from d 0 to 54 was decreased for heifers fed microalgae ($P = 0.006$) compared to heifers not fed microalgae. Dry matter intake from d 55 to 98, d 99 to 180, or overall did not differ between treatments ($P \geq 0.31$).

2.4.2 Reproductive performance

Although heifers supplemented with microalgae had a 2.1 mm greater follicular diameter compared to cows fed the control supplement (Table 2.4), the size difference was not significant ($P = 0.12$). Follicular IGF-1 concentrations were greater in ALG compared to CON heifers ($P =$

0.03), but treatment did not have an effect (P = 0.62) on follicular estrogen concentration or reproductive tract scores. Conception to first AI (Table 2.4) did not differ between the CON and ALG heifers (CON = 59.1%, ALG = 54.6%, P = 0.67). No ALG heifers conceived during the second service, therefore when first and second AI conception rates were combined (Table 2.4), ALG heifers tended to have a lower conception rate compared to CON heifers (CON = 72.7%, ALG = 54.6%, P = 0.08). Overall breeding season conception rate (Table 2.4), including natural service, was decreased for ALG compared to CON heifers (CON = 93.2, ALG = 75.0%, P = 0.03).

2.4.3 Blood Assays

Heifers fed microalgae had decreased concentrations (P = 0.05) of glucose at hour 3 after feeding compared to heifers not fed microalgae (Figure 2.2). However, glucose concentration did not differ among treatment groups at any other time point ($P \geq 0.24$). Serum insulin concentrations (Figure 2.3) were decreased ($P \leq 0.04$) in animals fed microalgae at 0 and 6 h and tended to be lower at 9 h after feeding (P = 0.10). Serum insulin:glucose on day 180 (Figure 2.4) was decreased in heifers fed microalgae at all time points ($P \leq 0.04$) except 3 h after feeding (P = 0.75).

2.4.4 Subsequent growth and reproductive performance

Pre-calving and post-calving BW for bred heifers on day 364 did not differ between treatments ($P \geq 0.44$), but cow BW at weaning on day 550 (P = 0.10) and calf birth BW (P = 0.08) tended to be greater in the microalgae supplemented cows. Calf BW at weaning did not differ between treatments (P = 0.48). Treatment ended on d 180 and all heifers returned to control diets. Daily gain (Table 2.3) did not differ ($P \geq 0.25$) between the treatment and control cattle from d 180 to pre-calving, or from post-calving to weaning. Additionally, conception rates during the

second breeding season as 2 yr olds (Table 2.4) were lower for heifers fed microalgae during the study compared to CON heifers (CON =86.7%, ALG =51.9%, $P = 0.009$).

2.5 Discussion

The decrease in DMI in ALG heifers compared to CON in the first period (d 0 - d 54) of the current study was likely caused by decreased palatability of microalgae due to smell, greater fat intake affecting rumen function, or the intake of omega-3 fatty acids. The goal was to provide the target 100 g/d. Decreases in DMI have also been observed in dairy cattle fed 150 to 900 g/d of microalgae (Franklin et al., 1999; Vanbergue et al., 2018), in steers fed 100 g/d of microalgae (Carvalho et al., 2018), and in wethers fed 2% of the diet DM as microalgae (Diaz et al., 2017). Feeding 100 g/d or less of microalgae still increases milk n-3 content, but did not decrease intake (Vahmani et al., 2013; Moran et al., 2018; Till et al., 2019). In addition, when up to 224 g/d of microalgae oil was fed to dairy cows, dry matter intake was unaffected and milk yield actually increased (Stamey et al., 2012), suggesting that something other than the oil or fatty acids are negatively impacting performance when microalgae is supplemented to cattle.

Heifer daily gain during the breeding season (day 55 to 98), heifer BW on day 98, 180, and at weaning, and calf birth BW were greater for the microalgae treatment. This is consistent with work in pigs, where Abril et al. (2003), observed an overall increase in ADG in growing barrows fed increasing amounts of DHA rich Schizochytrium microalgae for the last 120 days prior to slaughter. However, other studies have not found impacts on BW or ADG in feedlot cattle (Stokes et al., 2016; Carvalho et al., 2018), dairy cows (Till et al., 2019) or sheep (Meale et al., 2014). The increase in ADG in the present study could be a result of a shift in metabolism as a result of increased PUFA and n-3 intake. Long-chain n-3 PUFAs have been reported to improve insulin sensitivity in cattle (Cartiff et al., 2013). Improved growth in the current study may be a result of

changes in insulin production and glucose uptake. The fact that insulin and glucose were decreased in the present study indicates that dietary microalgae may have shifted energy utilization in tissues from glucose to fat, thus sparing glucose and impacting its uptake into tissues. Insulin is responsible for stimulating glucose utilization and promoting lipid and glycogen storage in tissues (Chaiyabutr et al., 2007). When steers were abomasally infused with fish oil, which is high in DHA and EPA, insulin sensitivity improved and protein breakdown and amino acid oxidation decreased (Gingras et al., 2007). Carvalho et al. (2018) observed that Aurantiochytrium microalgae increased blood glucose and decreased blood insulin concentrations during a glucose tolerance test. Fish oil was also reported to increase blood glucose in dairy cows (Kupczynski et al., 2012) and decrease circulating insulin in rats (Chicco et al., 1996; D'Alessandro et al., 2002). Previous studies in rats have demonstrated that dietary fish oil can promote lipid oxidation in tissues (Baker et al., 2000).

In the current study, season-long conception rates were significantly decreased in cattle supplemented with microalgae. In contrast, Sinedino et al. (2017) fed 100g/d of Schizochytrium microalgae containing 10% DHA from 27 to 147 d post-partum to primiparous and multiparous dairy cows and reported an increase in estrous activity compared to cows not fed microalgae. Cyclicity and pregnancy rates were increased in primiparous females fed microalgae, but no difference was observed for multiparous cattle supplemented with microalgae. Sinedino et al. (2017) also reported that microalagae decreased the number of days to pregnancy in both primiparous and multiparous cows, but the improvement was greater for primiparous cows. Furthermore, RTP4 gene expression, which is associated with placental development, immunomodulation and conceptus elongation, was upregulated in cows fed microalgae (Sinedino et al., 2017). Vlcek (2017) reported that feeding lactating dairy cows 100 g of Schizochytrium microalgae daily from day 47 to 99 of lactation did not affect the size of the pre-ovulatory follicle

at first or second synchronized estrus, although the size of the corpus luteum was larger in cows fed microalgae. Concentration of P_4, estradiol, insulin, NEFA, and cholesterol in pre-ovulatory follicular fluid did not differ between control and microalgae supplemented cows (Vlcek et al., 2017). Till et al. (2020) reported that supplementing lactating dairy cows with 100 g/d of Schizochytrium microalgae for the first 98 d of lactation had no effect on plasma concentrations of a $PGF_{2\alpha}$ metabolite, indicating that the n-3 fatty acids had no effect on $PGF_{2\alpha}$ synthesis. However, other n-3 rich oils, such as linseed oil (Dirandeh et al., 2013) and fish oil (Mattos et al., 2004) have decreased the $PGF_{2\alpha}$ metabolite in periparturient dairy cows. In addition, Hostens et al. (2011) observed that Schizochytrium microalgae supplementation increased the concentration of β-hydroxybutyric acid (BHBA) in follicular fluid, which in combination with a decrease in follicular glucose could be detrimental to oocyte development.

Increased IGF-1 concentrations in follicular fluid of heifers fed Schizochytrium in the present study compared to control heifers suggest that reproductive performance of microalgae fed heifers should have improved. Childs et al. (2008), fed increasing amounts of n-3 and DHA rich fish oil supplement to nulliparous crossbred heifers and reported increased plasma IGF-1 concentrations and an increase in n-3 fatty acids present in reproductive tissues. The study further helped formulate the hypothesis that increases in reproductive performance from fish oil supplementation could be due to an increase in CL diameter and subsequent increase in progesterone (Childs et al., 2008). Follicular IGF-1 plays a crucial role in selection and regulation of follicular dominance (Spicer et al., 1993). Both Insulin and IGF-1 increase follicular steroidogenesis as the follicles grow and develop (Webb et al., 1999; Spicer and Echternkamp, 1995 et al., Wathes et al., 1995). Greater follicular IGF-1 has been observed to decrease the time it takes for a follicle to reach competence and decreases postpartum interval (Beam et al., 1997),

whereas decreased concentrations of IGF-1 within the follicular fluid negatively affect ovulation and therefore conception rates (Taylor et al., 2004). However, IGF-1 produced in the follicle can have a negative effect on oocyte quality (Armstrong et al., 2003). This negative impact on oocyte quality could occur once the dominant follicle has become established because at this stage there is a shift in follicular requirements from growth hormone factors such as IGF-1 to gonadotropins, specifically LH (Webb et al., 2007). In fact, long-term exposure to IGF-1 within follicular fluid has been demonstrated to decreases oocyte quality (Armstrong et al., 2003). This aligns with the fact that first service conception rates in the current study did not differ, but later conception was decreased in heifers fed microalgae. This could mean that supplemental microalgae prolonged IGF-1 secretion and maximized follicular development, but also had a detrimental effect on the oocyte within. Although follicular diameter was decreased in heifers fed microalgae, it did not statistically differ from heifers not fed microalgae. Similar to the results observed in our current study, the majority of research (Burke et al., 1996; Petit et al., 2002; Robinson et al., 2002) has observed that follicular diameter is not affected by n-3 compared to n-6 fatty acid supplementation. However, supplementation of n-3 fatty acids have increased follicular diameter (Ambrose et al., 2006), and a decrease in follicular diameter has been observed in diets containing supplemental n-6 fatty acids (Homa et al., 1992).

Another reason that microalgae in the current study decreased conception rates may be because of its significant eicosapentaenoic acid (EPA, 20:5n-3) content. Two-series prostaglandins, including $PGF_{2\alpha}$, are derived from arachidonic acid (20:4n-6), which is a 20-carbon n-6 fatty acid that is elongated from linoleic acid (18:2n-6). Prostaglandins derived from EPA, including $PGF_{3\alpha}$, are considered “3-series” PGs and are believed to be less inflammatory. The 2-series prostaglandins along with the 3-series are synthesized using a common enzyme. Dietary

supplements rich in n-3 PUFA increase the synthesis of 3-series PG that competitively inhibit synthesis of 2-series PG, of which PGF2α is the major driver of luteolysis. The majority of early embryo loss during the initial stages of pregnancy establishment can be directly correlated with the embryos inability to inhibit synthesis of PGF2α around the time of maternal recognition on about d 15 post-breeding (Chagas et al., 2007). When the ratio of series 2 and series 3 prostaglandins is altered, the timing of luteolytic responses are changed which results in increased production of progesterone, hypothetically extending the functionality and lifespan of the CL (Kim et al., 2016). While an increased lifespan for the CL increases embryo survival, extends the longevity of progesterone production (Petit et al., 2009), and is good for maintenance of pregnancy (Otto et al., 2014), it can inhibit the development of new follicles and delay ovulation and behavioral estrus. This may explain why second service AI conception rates in the current study were negatively affected.

The fact that microalgae supplementation did not affect follicular estrogen concentrations in the present study suggests that sexual behavior and receptivity may not have been impacted. Estrogen is a sexual promoter that is produced within the follicle and is responsible for regulating sexual behavior along with regulation of the hypothalamic and anterior pituitary function (Senger., 2012). Estradiol is the primary regulator of the LH surge from the anterior pituitary. These two hormones are responsible for the increase in frequency of LH pulses during the follicular phase, which is a primary factor in the development of dominant follicles on the ovary (Kinder et al., 1996).

The dramatic decrease in conception rates the following breeding season in the current study was surprising. However, because dietary treatments took place during heifers growing phase, it is likely that n-3 fatty acids were deposited in fat reserves (BCS increased during the

study). When cows mobilized body fat after calving (BCS decreased from day 180 to weaning), circulating n-3 fatty acids may have increased, resulting in $PGF_{2\alpha}$ inhibition and/or excessive IGF-1 concentration in follicular fluid.

Results from the current study indicate that inclusion of Schizochytrium microalgae in the diet of nulliparous beef heifers during the breeding season improves body weight and calf birth weights, but negatively impacted reproduction. The decrease in conception rates during this study's supplemental treatment period, as well as during the following year's breeding season, suggests a negative impact on profitability and overall herd production. This impact not only affects short-term performance of the heifers, but also their long-term productivity within the herd. Inhibition of PGF2α secretion with dietary omega-3 fatty acids, in theory, should have resulted in greater conception and pregnancy rates due to a decrease in embryo loss due to the embryos inability to inhibit PGF2α independently (Petit et al., 2009). Future research is necessary to determine the appropriate ratio of n-3 and n-6 fatty acids in the diet, optimal duration of supplementation, optimal time during the reproductive cycle, and if there is an impact of age or parity on animal response to microalgae supplementation.

2.6 References

Abril, R., Garrett, J., Zeller, S. G., Sander, W. J., & Mast, R. W. (2003). Safety assessment of DHA-rich microalgae from Schizochytrium sp. Part V: target animal safety/toxicity study in growing swine. *Regulatory Toxicology and Pharmacology*, *37*(1), 73–82. https://doi.org/https://doi.org/10.1016/S0273-2300(02)00030-2

Ambrose, D. J., Kastelic, J. P., Corbett, R., Pitney, P. A., Petit, H. V, Small, J. A., & Zalkovic, P. (2006). Lower Pregnancy Losses in Lactating Dairy Cows Fed a Diet Enriched in α-Linolenic Acid. *Journal of Dairy Science*, *89*(8), 3066–3074. https://doi.org/https://doi.org/10.3168/jds.S0022-0302(06)72581-4

Armstrong, J. D., Goodall, E. A., Gordon, F. J., Rice, D. A., & McCaughey, W. J. (1990). The effects of levels of concentrate offered and inclusion of maize gluten or fish meal in the

concentrate on reproductive performance and blood parameters of dairy cows. *Animal Science*, *50*(1), 1–10. https://doi.org/DOI: 10.1017/S0003356100004414

Baker, P. W., & Gibbons, G. F. (2000). Effect of dietary fish oil on the sensitivity of hepatic lipid metabolism to regulation by insulin. *Journal of Lipid Research*, *41*(5), 719–726.

Beam, S. W., & Butler, W. R. (1997). Energy Balance and Ovarian Follicle Development Prior to the First Ovulation Postpartum in Dairy Cows Receiving Three Levels of Dietary Fat. *Biology of Reproduction*, *56*(1), 133–142. https://doi.org/10.1095/biolreprod56.1.133

Bellows, R. A., Grings, E. E., Simms, D. D., Geary, T. W., & Bergman, J. W. (2001). Effects of Feeding Supplemental Fat During Gestation to First-Calf Beef Heifers[1]. *The Professional Animal Scientist*, *17*(2), 81–89. https://doi.org/10.15232/S1080-7446(15)31602-8

Burke, J. M., Staples, C. R., Risco, C. A., De La Sota, R. L., & Thatcher, W. W. (1997). Effect of Ruminant Grade Menhaden Fish Meal on Reproductive and Productive Performance of Lactating Dairy Cows1. *Journal of Dairy Science*, *80*(12), 3386–3398. https://doi.org/https://doi.org/10.3168/jds.S0022-0302(97)76314-8

Cartiff, S. E., Fellner, V., & Eisemann, J. H. (2013). Eicosapentaenoic and docosahexaenoic acids increase insulin sensitivity in growing steers1. *Journal of Animal Science*, *91*(5), 2332–2342. https://doi.org/10.2527/jas.2012-5694

Carvalho, J. R. R., Brennan, K. M., Ladeira, M. M., & Schoonmaker, J. P. (2018). Performance, insulin sensitivity, carcass characteristics, and fatty acid profile of beef from steers fed microalgae1. *Journal of Animal Science*, *96*(8), 3433–3445. https://doi.org/10.1093/jas/sky210

Chagas, L. M., Bass, J. J., Blache, D., Burke, C. R., Kay, J. K., Lindsay, D. R., Lucy, M. C., Martin, G. B., Meier, S., & Rhodes, F. M. (2007). Invited review: New perspectives on the roles of nutrition and metabolic priorities in the subfertility of high-producing dairy cows. *Journal of Dairy Science*, *90*(9), 4022–4032.

Chaiyabutr, N., Faulkner, A., & Peaker, M. (1983). Effects of exogenous glucose on glucose metabolism in the lactating goat in vivo. *British Journal of Nutrition*, *49*(1), 159–165. https://doi.org/DOI: 10.1079/BJN19830021

Chicco, A., D'Alessandro, M. E., Karabatas, L., Gutman, R., & Lombardo, Y. B. (1996). Effect of moderate levels of dietary fish oil on insulin secretion and sensitivity, and pancreas insulin content in normal rats. *Annals of Nutrition and Metabolism*, *40*(2), 61–70.

Childs, S., Hennessy, A. A., Sreenan, J. M., Wathes, D. C., Cheng, Z., Stanton, C., Diskin, M. G., & Kenny, D. A. (2008). Effect of level of dietary n-3 polyunsaturated fatty acid supplementation on systemic and tissue fatty acid concentrations and on selected reproductive variables in cattle. *Theriogenology*, *70*(4), 595–611. https://doi.org/https://doi.org/10.1016/j.theriogenology.2008.04.002

Cooper, S. L., Sinclair, L. A., Wilkinson, R. G., Hallett, K. G., Enser, M., & Wood, J. D. (2004). Manipulation of the n-3 polyunsaturated fatty acid content of muscle and adipose tissue in lambs1. *Journal of Animal Science*, *82*(5), 1461–1470. https://doi.org/10.2527/2004.8251461x

D'Alessandro, M. E., Lombardo, Y. B., & Chicco, A. (2002). Effect of dietary fish oil on insulin sensitivity and metabolic fate of glucose in the skeletal muscle of normal rats. *Annals of Nutrition and Metabolism*, *46*(3–4), 114–120.

Díaz, M. T., Pérez, C., Sánchez, C. I., Lauzurica, S., Cañeque, V., González, C., & De La Fuente, J. (2017). Feeding microalgae increases omega 3 fatty acids of fat deposits and muscles in light lambs. *Journal of Food Composition and Analysis*, *56*, 115–123. https://doi.org/https://doi.org/10.1016/j.jfca.2016.12.009

Dirandeh, E., Towhidi, A., Zeinoaldini, S., Ganjkhanlou, M., Ansari Pirsaraei, Z., & Fouladi-Nashta, A. (2013). Effects of different polyunsaturated fatty acid supplementations during the postpartum periods of early lactating dairy cows on milk yield, metabolic responses, and reproductive performances. *Journal of Animal Science*, *91*(2), 713–721.

Franklin, S. T., Martin, K. R., Baer, R. J., Schingoethe, D. J., & Hippen, A. R. (1999). Dietary Marine Algae (Schizochytrium sp.) Increases Concentrations of Conjugated Linoleic, Docosahexaenoic and Transvaccenic Acids in Milk of Dairy Cows. *The Journal of Nutrition*, *129*(11), 2048–2054. https://doi.org/10.1093/jn/129.11.2048

Gingras, A., White, P. J., Chouinard, P. Y., Julien, P., Davis, T. A., Dombrowski, L., Couture, Y., Dubreuil, P., Myre, A., & Bergeron, K. (2007). Long-chain omega-3 fatty acids regulate bovine whole-body protein metabolism by promoting muscle insulin signalling to the Akt–mTOR–S6K1 pathway and insulin sensitivity. *The Journal of Physiology*, *579*(1), 269–284.

Gulliver, C. E., Friend, M. A., King, B. J., & Clayton, E. H. (2012). The role of omega-3 polyunsaturated fatty acids in reproduction of sheep and cattle. *Animal Reproduction Science*, *131*(1–2), 9–22.

Harel, M., Koven, W., Lein, I., Bar, Y., Behrens, P., Stubblefield, J., Zohar, Y., & Place, A. R. (2002). Advanced DHA, EPA and ArA enrichment materials for marine aquaculture using single cell heterotrophs. *Aquaculture*, *213*(1), 347–362. https://doi.org/https://doi.org/10.1016/S0044-8486(02)00047-9

Homa, S. T., & Brown, C. A. (1992). Changes in linoleic acid during follicular development and inhibition of spontaneous breakdown of germinal vesicles in cumulus-free bovine oocytes. *Reproduction*, *94*(1), 153–160. https://doi.org/10.1530/jrf.0.0940153

Hostens, M., Fievez, V., Vlaeminck, B., Buyse, J., Leroy, J., Piepers, S., De Vliegher, S., & Opsomer, G. (2011). The effect of marine algae in the ration of high-yielding dairy cows during transition on metabolic parameters in serum and follicular fluid around parturition.

Journal of Dairy Science, *94*(9), 4603–4615. https://doi.org/https://doi.org/10.3168/jds.2010-3899

JG, A. D. G. G., & Webb, R. (2003). Interactions between nutrition and ovarian activity in cattle: physiological cellular and molecular mechanisms. *Reproduction Supplement*, *61*, 403–414.

Kim, D. H., Lee, H. J., Amanullah, S. M., Adesogan, A. T., & Kim, S. C. (2016). Effects of dietary n-6/n-3 fatty acid ratio on nutrient digestibility and blood metabolites of H anwoo heifers. *Animal Science Journal*, *87*(1), 46–53.

Kinder, J. E., Kojima, F. N., Bergfeld, E. G. M., Wehrman, M. E., & Fike, K. E. (1996). Progestin and estrogen regulation of pulsatile LH release and development of persistent ovarian follicles in cattle. *Journal of Animal Science*, *74*(6), 1424–1440. https://doi.org/10.2527/1996.7461424x

Kupczyński, R., Kuczaj, M., Szołtysik, M., & Stefaniak, T. (2012). Influence of fish oil, palm oil and glycerol on milk fatty acid composition and metabolism in cows during early lactation. *Archiv Tierzucht*, *55*(6), 540–551.

Lopes, C. N., Scarpa, A. B., Cappellozza, B. I., Cooke, R. F., & Vasconcelos, J. L. M. (2009). Effects of rumen-protected polyunsaturated fatty acid supplementation on reproductive performance of Bos indicus beef cows1. *Journal of Animal Science*, *87*(12), 3935–3943. https://doi.org/10.2527/jas.2009-2201

Mattos, R., Staples, C. R., & Thatcher, W. W. (2000). Effects of dietary fatty acids on reproduction in ruminants. *Reviews of Reproduction*, *5*, 38–45.

Meale, S. J., Chaves, A. V, He, M. L., & McAllister, T. A. (2014). Dose–response of supplementing marine algae (Schizochytrium spp.) on production performance, fatty acid profiles, and wool parameters of growing lambs1. *Journal of Animal Science*, *92*(5), 2202–2213. https://doi.org/10.2527/jas.2013-7024

Moran, C. A., Morlacchini, M., Keegan, J. D., & Fusconi, G. (2018). The effect of dietary supplementation with Aurantiochytrium limacinum on lactating dairy cows in terms of animal health, productivity and milk composition. *Journal of Animal Physiology and Animal Nutrition*, *102*(2), 576–590.

National Academies of Sciences and Medicine, E. (2016). NASEM (2016). *Use of Metabolomics to Advance Research on Environmental Exposures and the Human Exposome. Available Online at: Https://Www. Nap. Edu/Read/23414*.

Nute, G. R., Richardson, R. I., Wood, J. D., Hughes, S. I., Wilkinson, R. G., Cooper, S. L., & Sinclair, L. A. (2007). Effect of dietary oil source on the flavour and the colour and lipid stability of lamb meat. *Meat Science*, *77*(4), 547–555. https://doi.org/https://doi.org/10.1016/j.meatsci.2007.05.003

Otto, J. R., Freeman, M. J., Malau-Aduli, B. S., Nichols, P. D., Lane, P. A., & Malau-Aduli, A. E. O. (2014). Reproduction and fertility parameters of dairy cows supplemented with omega-3 fatty acid-rich canola oil. *Annual Research & Review in Biology*, *4*, 1611–1636.

Pate, J. L., Johnson-Larson, C. J., & Ottobre, J. S. (2012). Life or death decisions in the corpus luteum. *Reproduction in Domestic Animals*, *47*, 297–303.

Petit, H. V, & Twagiramungu, H. (2006). Conception rate and reproductive function of dairy cows fed different fat sources. *Theriogenology*, *66*(5), 1316–1324. https://doi.org/https://doi.org/10.1016/j.theriogenology.2006.04.029

Petit, H. V, Gagnon, N., & Palin, M.-F. (2009). Influence of Fatty Acids on Reproduction and Immune Function in Ruminants. *30 Th Western Nutrition Conference*, 139.

Robinson, R. S., Pushpakumara, P. G. A., Cheng, Z., Peters, A. R., Abayasekara, D. R. E., & Wathes, D. C. (2002). Effects of dietary polyunsaturated fatty acids on ovarian and uterine function in lactating dairy cows. *REPRODUCTION-CAMBRIDGE-*, *124*(1), 119–131.

Sampels, S., Turner, T., Öström, Å., & Pickova, J. (2010). Effects of α-linolenic acid and eicosapentaenoic acid from linseed and algae, respectively, on reindeer (Rangifer tarandus tarandus L.) muscle fatty acid composition. *Acta Agriculturae Scandinavica, Section A — Animal Science*, *60*(3), 175–186. https://doi.org/10.1080/09064702.2010.523092

Santos, J. E. P., Bilby, T. R., Thatcher, W. W., Staples, C. R., & Silvestre, F. T. (2008). Long chain fatty acids of diet as factors influencing reproduction in cattle. *Reproduction in Domestic Animals*, *43*, 23–30.

Santos, J. E. P., Greco, L. F., Garcia, M., Thatcher, W. W., Staples, C. R., & Santos, J. E. P. (2013). The role of specific fatty acids on dairy cattle performance and fertility. *Florida Ruminant Nutrition Symposium, University of Florida, Gainesville, FL*, 74–88.

Senger, P. L. (2012). *Pathways to pregnancy & parturition*.

Silvestre, F. T., Carvalho, T. S. M., Crawford, P. C., Santos, J. E. P., Staples, C. R., Jenkins, T., & Thatcher, W. W. (2011). Effects of differential supplementation of fatty acids during the peripartum and breeding periods of Holstein cows: II. Neutrophil fatty acids and function, and acute phase proteins. *Journal of Dairy Science*, *94*(5), 2285–2301.

Silvestre, F. T., Carvalho, T. S. M., Francisco, N., Santos, J. E. P., Staples, C. R., Jenkins, T. C., & Thatcher, W. W. (2011). Effects of differential supplementation of fatty acids during the peripartum and breeding periods of Holstein cows: I. Uterine and metabolic responses, reproduction, and lactation. *Journal of Dairy Science*, *94*(1), 189–204. https://doi.org/https://doi.org/10.3168/jds.2010-3370

Sinedino, L. D. P., Honda, P. M., Souza, L. R. L., Lock, A. L., Boland, M. P., Staples, C. R., Thatcher, W. W., & Santos, J. E. P. (2017). Effects of supplementation with docosahexaenoic acid on reproduction of dairy cows. *Reproduction*, *153*(5), 707–723.

Spicer, L. J., Alpizar, E., & Echternkamp, S. E. (1993). Effects of insulin, insulin-like growth factor I, and gonadotropins on bovine granulosa cell proliferation, progesterone production, estradiol production, and(or) insulin-like growth factor I production in vitro1. *Journal of Animal Science*, *71*(5), 1232–1241. https://doi.org/10.2527/1993.7151232x

Spicer, L. J., & Echternkamp, S. E. (1995). The ovarian insulin and insulin-like growth factor system with an emphasis on domestic animals. *Domestic Animal Endocrinology*, *12*(3), 223–245. https://doi.org/https://doi.org/10.1016/0739-7240(95)00021-6

Stamey, J. A., Shepherd, D. M., de Veth, M. J., & Corl, B. A. (2012). Use of algae or algal oil rich in n-3 fatty acids as a feed supplement for dairy cattle. *Journal of Dairy Science*, *95*(9), 5269–5275. https://doi.org/https://doi.org/10.3168/jds.2012-5412

Stokes, R. S., Loy, D. D., & Hansen, S. L. (2016). Effects of increased inclusion of algae meal on finishing steer performance and carcass characteristics. *Journal of Animal Science*, *94*(2), 687–696.

Taylor, V. J., Cheng, Z., Pushpakumara, P. G. A., Wathes, D. C., & Beever, D. E. (2004). Relationships between the plasma concentrations of insulin-like growth factor-I in dairy cows and their fertility and milk yield. *Veterinary Record*, *155*(19), 583 LP – 588. https://doi.org/10.1136/vr.155.19.583

Till, B. E., Huntington, J. A., Kliem, K. E., Taylor-Pickard, J., & Sinclair, L. A. (2020). Long term dietary supplementation with microalgae increases plasma docosahexaenoic acid in milk and plasma but does not affect plasma 13,14-dihydro-15-keto PGF2α concentration in dairy cows. *Journal of Dairy Research*, *87*(1), 14–22. https://doi.org/DOI: 10.1017/S002202991900102X

Vahmani, P., Fredeen, A. H., & Glover, K. E. (2013). Effect of supplementation with fish oil or microalgae on fatty acid composition of milk from cows managed in confinement or pasture systems. *Journal of Dairy Science*, *96*(10), 6660–6670. https://doi.org/https://doi.org/10.3168/jds.2013-6914

Vanbergue, E., Peyraud, J.-L., & Hurtaud, C. (2018). Effects of new n-3 fatty acid sources on milk fatty acid profile and milk fat properties in dairy cows. *Journal of Dairy Research*, *85*(3), 265–272.

Vlcek, M., Andrlikova, M., Barbato, O., Bina, V., Boland, M. P., Dolezel, R., Lopatarova, M., & Cech, S. (2017). Supplementation of dairy cows with docosahexaenoic acid did not affect ovarian activity. *Czech Journal of Animal Science*, *62*(11), 457–465.

Wagner, J. J., Lusby, K. S., Oltjen, J. W., Rakestraw, J., Wettemann, R. P., & Walters, L. E. (1988). Carcass composition in mature Hereford cows: Estimation and effect on daily metabolizable energy requirement during winter. *Journal of Animal Science*, *66*(3), 603–612.

Wathes, D. C., Perks, C. M., Davis, A. J., & Denning-Kendall, P. A. (1995). Regulation of Insulin-Like Growth Factor-I and Progesterone Synthesis by Insulin and Growth Hormone in the Ovine Ovary1. *Biology of Reproduction*, *53*(4), 882–889. https://doi.org/10.1095/biolreprod53.4.882

Webb, R., & Campbell, B. K. (2007). Development of the dominant follicle: mechanisms of selection and maintenance of oocyte quality. *Society of Reproduction and Fertility Supplement*, *64*, 141.

Webb, R., Gosden, R. G., Telfer, E. E., & Moor, R. M. (1999). Factors affecting folliculogenesis in ruminants. *Animal Science*, *68*(2), 257–284.

Zachut, M., Arieli, A., Lehrer, H., Argov, N., & Moallem, U. (2008). Dietary unsaturated fatty acids influence preovulatory follicle characteristics in dairy cows. *REPRODUCTION*, *135*(5), 683–692. https://doi.org/10.1530/REP-07-0556

Zeron, Y., Sklan, D., & Arav, A. (2002). Effect of polyunsaturated fatty acid supplementation on biophysical parameters and chilling sensitivity of ewe oocytes. *Molecular Reproduction and Development*, *61*(2), 271–278. https://doi.org/10.1002/mrd.1156

Table 2.1. Diet composition

	CON	ALG
Corn silage	32.0	32.0
Haylage	37.0	37.0
Ryelage	15.8	15.8
Corn	4.0	-----
Dried distillers grains with solubles	10.0	6.5
Algae supplement[1]	-----	7.5
Vitamin/Mineral Supplement[2]	1.2	1.2
Diet composition (DM basis)[3]		
Dry matter	49.2	49.2
Crude protein, %	12.0	12.0
NEm[4], Mcal/kg.	1.39	1.39
NEg[4], Mcal/kg.	0.79	0.79
Neutral detergent fiber	41.6	41.6
Fat	3.9	4.6
Calcium, %	0.61	0.60
Phosphorus, %	0.33	0.33
Potassium, %	1.85	1.85
Sulfur, %	0.19	0.18
Fatty acids (g/100 g)	1.22	1.39
<C16:0	1.22	1.39
C16:0	21.31	21.30
C18:0	6.03	5.95
C18:1 *cis*-9	18.18	17.76
C18:2 *cis*-9,12	29.62	28.95
C18:3 *cis*-9,12,15	16.74	16.70
Arachidonic acid (C20:4 *cis*-5,8,11,14)	0.000	0.078
Eicosapentaenoic acid (C20:5 *cis*-5,8,11,14,17)	0.000	0.052
Docosohexaenoic acid (C22:6 cis-4,7,10,13,16,19)	0.000	0.517
Other fatty acids	5.70	6.10
SFA	32.59	32.67
MUFA	19.45	19.03
PUFA	46.76	47.09
n-3	16.74	17.33
n-6	29.82	29.52

[1]Algae Supplement was (DM basis) 40 % ground corn, 38% dried distillers grains with solubles, and 22% whole cell Schizochytrium species microalgae (DHA Gold; DSM Nutritionals; total fat content ≥ 40 %; minimum of 17% DHA)

[2]Vitamin/Mineral Supplement was17% Ca, 15% Na, 3% Mg, 2% K, 40 ppm Co, 200 ppm I, 3750 ppm Mn, 26 ppm Se, 5000 ppm Zn, 881,060 IU/kg vitamin A, 88,105 IU/kg vitamin D_3, 1762 IU/kg vitamin E

[3]Analyzed at Cumberland Valley Analytical Services (Waynesboro, PA)

[4]Calculated based on NASEM (2016)

Table 2.2. Effect of microalgae on body weight and body condition score

	No Algae	Algae	SEM	P-value
n[1]	44(32)	44(24)		
Heifer body weight, kg				
Day 0	426.3	428.1	1.85	0.50
Day 54	456.7	457.4	1.85	0.80
Day 98	465.2	470.7	1.85	0.06
Day 180[1]	507.5	516.7	2.79	0.03
Pre-calving (day 364)[1,2]	608.3	607.3	2.79	0.81
Post-calving (day 364)[1,2]	549	552.7	3.08	0.44
Weaning (day 550)[1,4]	563.6	573.3	3.92	0.10
Calf birth body weight[1,3]	33.9	35.3	0.45	0.08
Calf weaning weight (207 d)[4]	254.3	261.0	13.99	0.48
Body condition score				
Day 0	5.2	5.2	0.05	0.85
Day 54	5.3	5.5	0.05	0.1
Day 98[1]	5.4	5.4	0.06	0.63
Day 180[1]	5.6	5.5	0.06	0.8
Weaning (day 550)[2]	5.4	5.4	0.08	0.75

[1]Open heifers were removed on day 109
[2]Adjusted for gravid uterine weight (Ferrel, 1976). Post-calving weight $743e^{(0.02-0.0000143t)t}$
[3]Average calving day = 344
[4]n=22 algae; n=30 no algae

Table 2.3. Effects of microalgae on average daily gain and dry matter intake

	No Algae	Algae	SEM	P-value
n[1]	44(32)	44(24)		
Average daily gain, kg/d				
Day 0 to 54	0.56	0.54	0.034	0.68
Day 55 to 98	0.20	0.30	0.034	0.03
Day 99 to 180[1]	0.54	0.55	0.034	0.87
Day 0 to 180[1]	0.46	0.49	0.014	0.25
Day 180 to pre-calving (day 364)[1]	0.54	0.51	0.033	0.50
Post-calving to weaning (day 550)[2]	0.08	0.10	0.034	0.61
Dry matter intake, kg/d				
Day 0 to 54	6.2	5.9	0.05	0.006
Day 55 to 98	6.9	6.9	0.05	0.33
Day 99 to 180[1]	7.5	7.5	0.05	0.51
Day 0 to 180[1]	6.9	6.9	0.04	0.31

[1]Open heifers were removed on day 109
[2] n=22 algae; n=30 no algae

Table 2.4. Reproductive Performance

	No Algae	Algae	SEM	P-value
Follicular characteristics				
Diameter, mm	13.5	11.4	0.82	0.12
Insulin-like growth factor 1, pg/ml[1]	111.9	130.1	5.71	0.03
Estrogen, pg/ml[1]	150.1	176.0	36.26	0.62
Reproductive score	4.40	4.38	0.084	0.90
Conception rates				
First AI	59.1	54.6	-----	0.67
First and second AI	72.7	54.6	-----	0.08
Overall	93.2	75.0	-----	0.03
Year 2 overall	86.7	51.9	-----	0.009

[1]IGF-1 and estrogen analyzed on follicular fluid aspirated on day 47

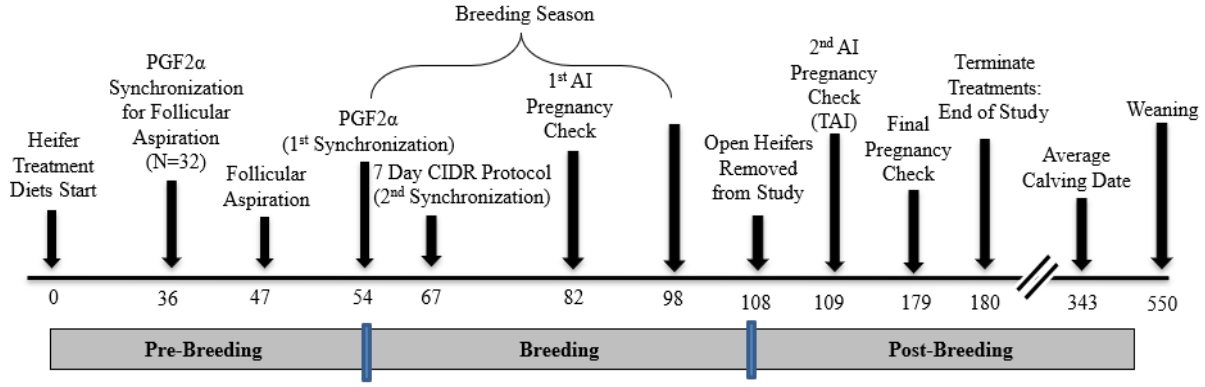

Figure 2.1. Timeline of treatment implementation and evaluations conducted. Treatments were initiated 54 d. prior to breeding in all heifers.

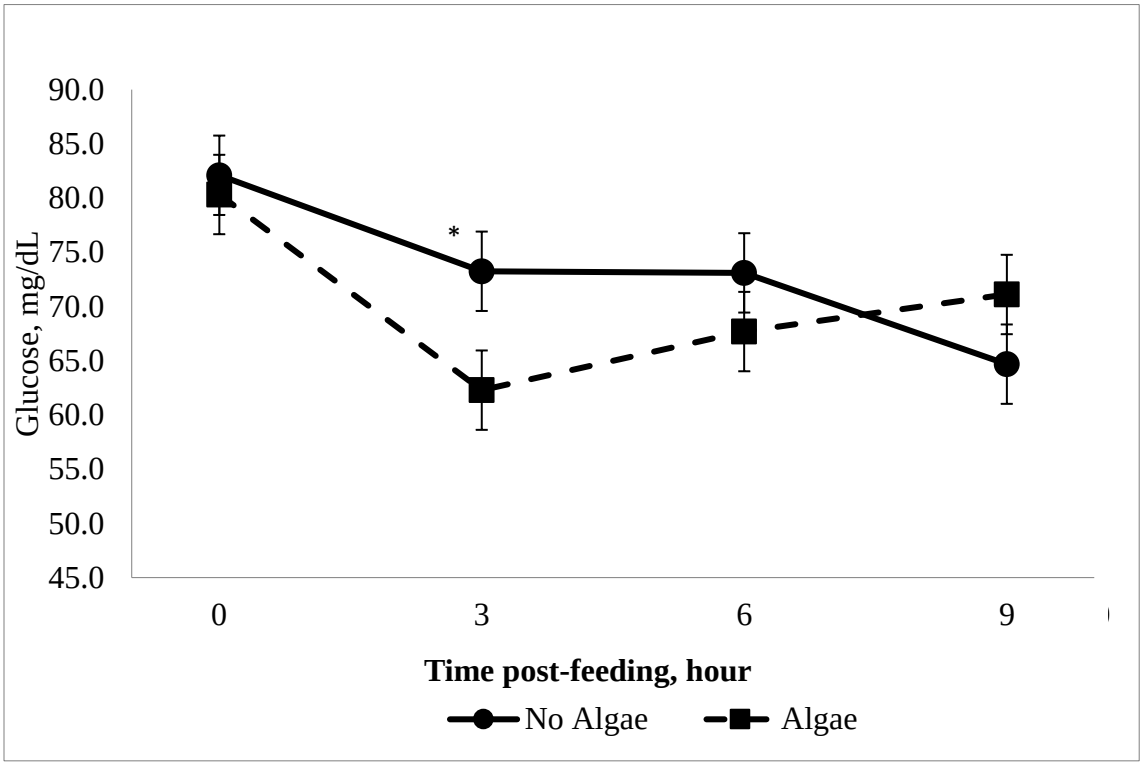

Figure 2.2. Effect of microalgae on serum glucose concentration on day 180. Treatment P = 0.49, treatment × time P = 0.02. The symbol (*) indicates a response ($P \leq 0.05$) of serum glucose to dietary microalgae inclusion.

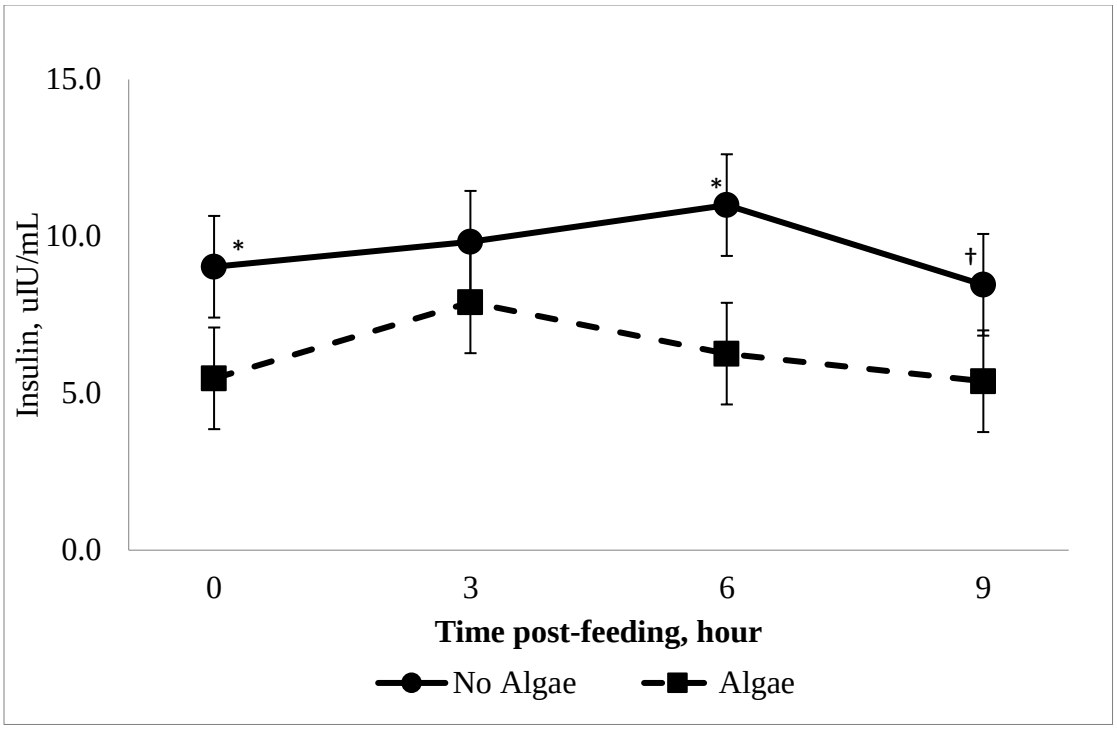

Figure 2.3. Effect of microalgae on serum insulin concentration on day 180. Treatment $P = 0.05$, treatment × time $P = 0.52$. The symbol (*) indicates a response ($P \leq 0.05$) and the symbol (†) indicates a response ($P = 0.10$) of serum insulin to dietary microalgae inclusion.

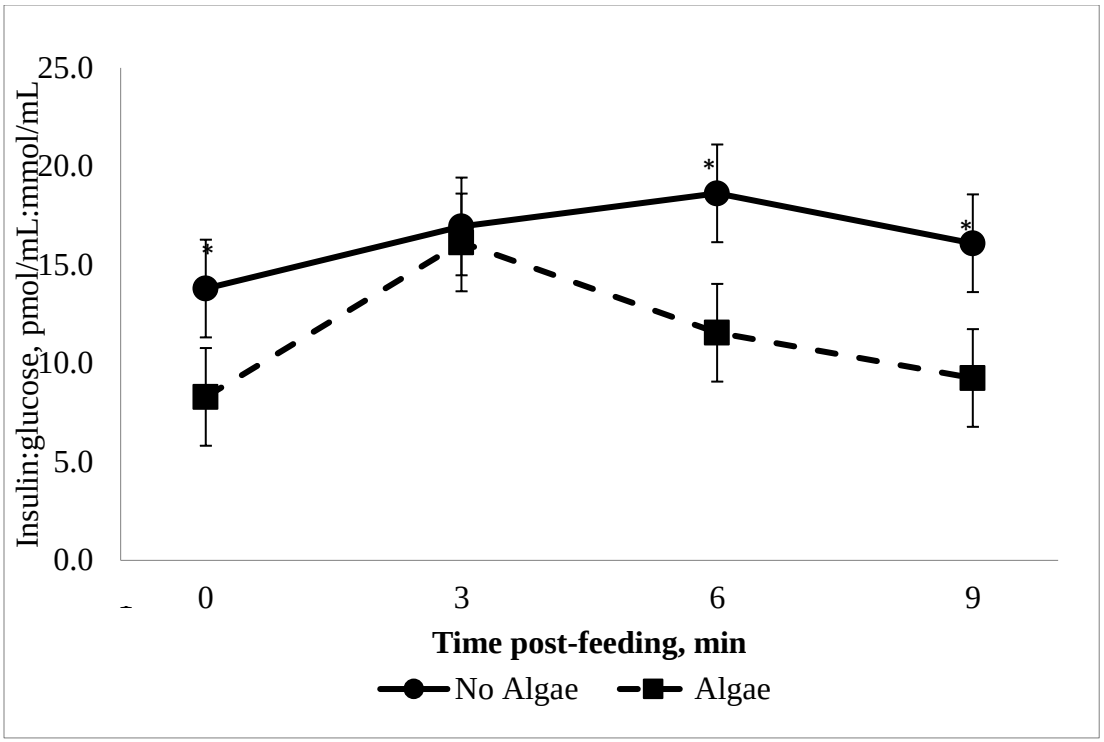

Figure 2.4. Effect of microalgae on serum insulin:glucose ratio on day 180. Treatment P = 0.002, treatment × time P = 0.29. The symbol (*) indicates a response ($P \leq 0.05$) of serum glucose to dietary microalgae inclusion.