Strategies to Improve Reproductive Efficiency in Cattle with Dietary Intervention

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SCHOLARLY ABSTRACT

This dissertation examines how reproductive efficiency can be improved through dietary intervention. The use of diet to enhance reproduction is theoretically a low-cost and low-input method that will co-align with reproductive technologies' beneficiary effects. Ideally, with improved nutrition intervention, these technologies will become more feasible and practical to producers. The first study examines the diet nutrient composition and its relative interactions on days to ovulation postpartum, overall pregnancy rates, and pregnancy at first AI in cattle. Most notably, the highest predicted overall pregnancy rate for multiparous cows was observed at high crude protein (CP), moderate non-structural carbohydrates (NSC), Low neutral detergent fiber (NDF) and high ether extract (EE). Additionally, the highest predicted pregnancy at first AI rate was when CP was high, moderate NSC, high NDF, and low EE. The second study examines the impact that a high starch diet may have on reproductive hormones, follicular growth, and pregnancy rates. The high starch diet tended to have a greater placental associated glycoprotein concentration, a greater number of large follicles on the day of breeding and a 20% numerically greater overall conception rate. The high fat diet did exhibit a greater number of medium sized follicles on the day of breeding. There were no observed differences in progesterone concentrations between the two treatment groups. The final study examines the effect that a higher starch diet might have on follicular development, CL growth and size, reproductive hormones, and insulin like growth factor binding proteins -2, -3, -4, -5, and pregnancy associated plasma protein A gene expression in granulosa cells. The experimental group that received the diet greater in starch exhibited a greater concentration of plasma IGF-1, greater number of large follicles on the dates that insemination would occur in a TAI situation, and a lower amount of days to reach a dominant follicle at the diameter of ≥ 10mm. Additionally, the high fat diet did exhibit a greater concentration of progesterone. Although, no statistical differences were observed in the granulosa cell gene expression and corpus luteum size. The feeding technique here may provide producers insight into non-expensive and straightforward ways to improve their reproductive efficiency and, ideally, adopt reproductive technologies in harmony with dietary intervention.

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GENERAL AUDIENCE ABSTRACT

Improving reproductive efficiency in both beef and dairy cows is essential to continually improve meat and milk production to meet the growing global demand for animal food products. In both beef and dairy operations, the goal is to produce one healthy calf per cow per year. However, each operation type faces unique challenges which, at present, often preclude achieving this goal. Intensive genetic selection for milk production has resulted in inadvertent selection against reproductive efficiency in the dairy industry. In the beef industry, the production conditions and tight profit margins mean that producers are often hesitant to use assisted reproductive technologies like estrus synchronization or artificial insemination, which may help improve reproductive efficiencies. On account of these challenges, there is a clear need for supportive tools and technologies which can be applied within the beef and dairy industries to improve the reproductive efficiencies of these production systems.

One possible strategy that could be employed to improve reproductive efficiency within the beef and dairy industries is nutritional manipulation. Although there are mixed results within the literature regarding how dietary manipulation can be leveraged to affect reproductive outcomes, feed supplementation is typically a strategy readily leveraged by beef and dairy producers for various production outcomes. As such, dietary manipulation may be a promising method to improve reproductive efficiency, assuming discrepancies among studies within the literature can be reconciled to provide feeding recommendations to improve reproduction. One strategy to evaluate commonalities among literature data is the use of meta-analysis. Our first study leveraged meta-analysis to evaluate how diet composition and its interactions with parity, genetics, and housing situation affect reproductive outcomes. This study found that reproduction is affected by complex interactions among diet, management, genetics, and time. Thus, leveraging nutrition to improve reproductive efficiency will require precise identification of the correct supplement for the correct animals, based on their age and management conditions. Experimental work to confirm the efficacy of different nutritional modulation strategies will be essential to confirm such strategies before widespread use. Rather than experimentally evaluating an exhaustive listing of strategies to improve reproductive efficiency, confirmation experiments will be more efficient if they evaluate dietary approaches that appeared successful under most conditions simulated in the meta-analysis.

In our meta-analysis, supplementation of non-structural carbohydrates (NSC) and ether extract (EE) fairly consistently improved reproductive outcomes when compared with crude protein (CP) and neutral detergent fiber (NDF) supplementation, which had more mixed results. Current literature on these feed supplements is often confounded with energy intake, meaning that it is difficult to discern whether the consistency in response reflects consistent benefit of added energy or unique benefits of these energy-providing substrates. As such, we designed an experiment to evaluate the effect of isoenergetic supplementation of EE and NSC within the diets of grazing Angus crossbred beef heifers to evaluate the effects on reproductive outcomes.

Responses of interest included progesterone concentrations, pregnancy rates, placental-associated glycoprotein concentrations, and follicle size and number. Supplementation of NSC resulted in lower plasma progesterone and a significantly lower number of medium follicles on the day of breeding, relative to supplementation with fat. However, heifers supplemented with NSC also had elevated placental-associated glycoprotein concentrations, numerically increased conception rates, and a greater number of large follicles on the day of breeding when compared with the heifers supplemented with fat. As such, the data suggest the NSC diet promoted the maturation of large follicles, which may support improved conception rates. The EE diet promoted elevated progesterone concentrations, but did not appear to support the maturation of medium to large follicles, likely accounting for the numerically lower conception rates. Because heifers are well acknowledged to differ from cows due to their energy requirements, it is useful to also evaluate the efficacy of these dietary treatments in mature cows to assess the consistency of responses.

Toward that goal, the objective of our second study was to compare supplementation of high NSC or high EE feeds to mixed-breed, non-pregnant, multiparous beef cows when evaluating reproductive outcomes. The results of these supplementation strategies were similar to those identified within the heifer trial. The group consuming the high EE supplement had elevated plasma progesterone concentrations. The group consuming the high NSC supplement had a greater number of large follicles on the days of GnRH administration. There were no differences in gene expression of aspirated granulosa cells between the two groups. The consistency of results between the heifer and cow studies suggest that supplementation with high NSC or high fat supplements may consistently affect cows, irrespective of physiological stage. As such, further work to titrate the minimum required supplementation levels to assess the possible economic returns of this approach to optimizing reproductive efficiency.

Our studies concluded that reproductive efficiency can be enhanced through dietary manipulation in a manner independent of dietary energy intake. Additionally, the utilization of a propiogenic precursor such as a high NSC diet appears to support the maturation of follicles in cattle utilizing a timed artificial insemination estrus synchronization protocol, likely leading to improved pregnancy rates. As such, assessment of NSC supplementation for reproductive modulation may be a productive line of future work.

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CHAPTER 1

GENERAL INTRODUCTION

There is an increasing amount of pressure on livestock producers to continually improve production efficiencies in response to the global grand challenge of sustainably feeding the growing global population. Global population is expected to reach 9.8 billion by 2050 (United Nations, 2015). This population growth occurs simultaneously with decreased available land for agricultural use (Sands and Leimbach, 2003), increased per capita incomes (World Bank Group, 2013), and a greater proportion of the human population over 60 years of age (Blazhevska, 2019). Additionally, there is increasing concern about environmental impacts of livestock production. The challenges presented require the agriculture producer to find ways to become more efficient, producing more animal products with fewer inputs, and reduced environmental impact.

Improved production efficiency can come from improving animal health, enhancing nutrition and feed efficiency, improving genetic merit, and optimizing reproductive efficiency. Reproductive efficiency is a particularly interesting target because it reflects many of these other approaches – a cow will not reproduce unless she is healthy and has sufficient nutrient intakes. Additionally, it is through the process of reproduction that we dictate the effectiveness of strategies to improve genetic merit. As such, optimizing reproductive efficiency is an efficient target when working toward improving livestock systems productivity.

An ideal target for reproductive efficiency on both dairy and beef cattle operations is for every cow to produce one calf per year. However, reproductive failure is a leading source of economic and efficiency loss in the cow/calf agricultural sector (Toghiani et al., 2017), and in the dairy industry (Inchaisri et al., 2010). Reproductive inefficiency causes economic loss to the producer through a reduced number of calves born, increased breeding and labor costs, veterinary expenses, and a shorter reproductive utilization (Rahbar et al., 2016).

In response to the challenges associated with reproductive efficiency, and the importance of getting cows bred, several assisted reproductive technologies have been developed. Those include artificial insemination (AI), invitro fertilization (IVF), embryo transfer, estrus synchronization, and maintaining a defined breeding season (NAHMS, 2009). However, only 35% of all beef operations utilize any reproductive technologies (NAHMS, 2009). Some reasons for the slow adoption include; labor costs, time constraints, inadequate facilities, difficulty of implementation, technology costs, and because of claims that such technologies do not work (NAHMS, 2009). To improve the overall acceptance and usage of these technologies, their efficiency and ease-of-use needs to increase through low-cost and low-input methods.

One potential strategy to improve the effectiveness of assisted reproductive technologies is to completement technology use with dietary manipulation. Previous work has demonstrated that reproductive efficiency appears responsive to dietary nutrient concentrations, particularly protein (Blanchard et al., 1990) and fat (Funston, 2004). However, numerous practical aspects of the complementarity of nutritional modulation of reproductive outcomes have not been evaluated. For example, questions such as: (1) how long must a supplement be fed to significantly effect reproductive outcomes; (2) what types of supplements can be fed; and (3) how much must the diet change to see a shift in reproductive outcomes, remain unanswered. Ideally, diet manipulation to complement assisted reproductive technologies should be short-term, and be able to rely on low-cost feeds such as cracked corn. There is strong physiological evidence to support the idea that relatively low-cost and widely available feeds like corn grain can modulate the hypothalamic-pituitary-gonadal axis through the provision of propionate (Bedford et al., 2018), an end-product of rumen microbial fermentation of starch-rich substrate (Bauman et al., 1971), in fairly rapid response timeframes (<10 d). However, the relative responses attributable to energy type (i.e., propionate) versus simply improving energy balance have not been thoroughly evaluated. As such, there is a need to determine how reproductive efficiency metrics respond to short-term supplementation of feedstuffs differing in energy source, but with similar energy concentrations. In response to these gaps in knowledge, this dissertation evaluates the response of reproductive parameters to dietary supplements of differing nutrient and energy concentrations.

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CHAPTER 2

LITERATURE REVIEW

GLOBAL FOOD SECURITY AND ANIMAL REPRODUCTION

Global population growth

It is well documented (United Nations, 2015) that the global population is continuing to grow at a rapid rate, and is expected to reach 9.8 billion by 2050 (United Nations, 2015; Blazhevska, 2019). This population growth will dramatically increase food demand (Riggs et al., 2018) and create pressure within the agricultural communities to find adaptations to enhance food production productivity. In addition to population growth, there is also an increase in individuals' expected average lifespan. The population of people over the age of 60 is estimated to triple from 2019 to 2050 (Blazhevska, 2019). Another demographic shift expected to occur is the tripling of people's per capita incomes in developing countries between 2004 to 2030 (World Bank Group, 2013). This dramatic shift in per capita income is expected to increase animal-sourced food consumption (Cirera and Masset, 2010). Collectively, these population and demographic changes mean that demand for animal-source foods is likely to grow disproportionately as the population grows. In response to this increase in demand for animal source food, agriculturalists are faced with the challenge of increasing supply of these products accordingly.

Historical enhancements to cattle production

In the U.S., the past several decades of population growth provide an excellent example of the agricultural system's capacity to respond to increased supply. For example, U.S. beef production has risen dramatically from 6.67 million kg in 1960 to 10.1 million kg in 2019. Additionally, overall milk production has also increased from 55.8 million kg of milk produced in 1960 to 99.1 million kg produced in 2019 (USDA, QuickStats). The increase in milk production coincides with a drop in total dairy cows in the U.S.; although there were 19.5 million head of dairy cows in 1960, the national population was only 9.3 million head of dairy cows in 2019 (USDA, QuickStats). The combination of increased milk production and decreased cow numbers equates to over triple the annual milk production per cow (3,188 milk kg per head in 1960 vs 10,610 milk kg per head in 2019) (USDA, QuickStats). U.S. milk production increased from 14.7% to 15.6% of the global cattle milk market share from 2007 to 2017 (Capper and Cady, 2020). Although the herd size of the U.S. as a proportion of the global dairy herd declined slightly from 3.7% to 3.4% (UN, FAOSTAT), U.S. beef production nearly doubled between 1960 and 2019, increasing from 6.7 to 12.4 billion kg of beef. However, due to beef demand, there has been an increase in the total number of beef cows in the U.S. since 1960, with 26.3 million head in 1960 and 31.7 million head in 2019 (USDA, QuickStats). The increase in both beef production and the total number of animals still equates to over 130 additional kg of beef produced per mature cow in 2019 compared to 1960. Although these data suggest there is dramatic capacity with the agricultural system to enhance efficiency in response to shifting populations and population demographics, additional resource-use constraints expected to occur in response to population expansion may require additional shifts in livestock production systems.

Limitations in land and water availability

The dramatic increase in the global population is expected to result in land and water availability limitations for agricultural production (Sands and Leimbach, 2003; Sauer et al., 2010).

This resource availability limitation presents a significant challenge because these resource constraints, in combination with the increasing demand, will require additional efficiency improvements from the agricultural sector. Agriculture encompasses the most extensive use of land globally (Erb et al., 2018); however, future land accommodation for agriculture could be difficult due to already limited land availability and conflicting demands from population growth (Sauer et al., 2010). Agricultural production currently accounts for more than 70% of anthropogenic water usage (Bruinsma, 2017). Therefore, the agricultural demand for water is becoming increasingly competitive with other uses (e.g., human drinking water) as the population increases. The increase in global population and limitations in land and water availability are forcing agriculturalists to produce more food with fewer resources.

Although these trends in resource availability are alarming, they are not new. Global pasture availability in higher-income continents such as North America has declined by about 10% since 1961 (Blaustein-Rejto and Blomqvist, 2019). The decline in pasture availability is primarily attributed to urbanization and population growth (Vesterby et al., 1994), and most directly affects beef cattle production because most beef cattle production systems in the U.S. rely on pasture land. These data suggest that historical shifts in demand for land and water resources have pressured U.S. agriculture to enhance productivity and improve resource-use efficiency. Cattle can also have a negative effect on a pasture-based ecosystem, particularly when overstocked (Sitters et al., 2020). When overstocking occurs, grazing pressure leads to the replacement of palatable grasses with unpalatable ones, which impairs cattle productivity and negatively affects wildlife and other species in the surrounding ecosystem (Sitters et al., 2020). That said, when cattle are appropriately managed, grazing activities can be beneficial to the overall ecosystem and soil health (Young et al., 2018).

Greenhouse gas emissions

Another major challenge that must be considered when discussing food production expansion to address societal demand is greenhouse gas (GHG) emissions. A major cause of radiative forcing is from GHG emissions. It has been reported that as much as 19% of the increase in radiative forcing is due to CO_2 emitted since the preindustrial era (Rigby et al., 2014). These anthropogenic GHG emissions and their relative effect on the environment is of major concern to not only natural ecosystems, but also to human society (Cifuentes et al., 2001). The three primary GHG associated with agriculture are carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) (Liu and Greaver, 2009). At the global scale, electricity and heat production account for 25% of total emissions. Industry accounts for 21%, transportation for 14%, buildings for 6%, and other energy for 10% of total emissions. Agriculture, forestry, and other land usage account for 24% of total emissions (U.S. EPA, 2016). Of the agricultural emissions, over 40% are attributed to ruminant enteric methane emissions (Thoma et al., 2013). To determine radiative forcing equivalence among GHG, the standard method is to multiply the mass of each gas produced by a coefficient reflecting the radiative capacity of that gas relative to CO_2 (Hoegh-Guldberg et al., 2018). Under this calculation paradigm, methane gas is equivalent to roughly 24 times CO_2, which is part of why methane emissions from ruminant animals present such a challenge at the global scale. However, our understanding of how to account for equivalence among GHG is shifting in response to new studies which highlight the different half-life of each GHG (Cain et al., 2019). For example, CO_2 has an atmospheric lifespan that persists for thousands of years, while CH_4 only has an atmospheric lifespan of about a decade (U.S. EPA, 2015). The net result of the differing lifespan represents different opportunities to mitigate climate change by shifting CO_2 emissions when compared to shifting CH_4 emissions. Namely, in order to decrease radiative forcing from

CO_2 we must cease CO_2 emissions; however, in order to decrease radiative forcing from CH_4 we merely need to hold emissions constant. As such, Cain et al. (2019) conclude that reducing CH_4 emission, in particular, may be an extremely efficient means of addressing our global climate crisis. As such, ruminant agriculture is charged with finding strategies to dramatically decrease CH_4 emissions while continuing to meet global demand for meat and milk products.

Historically, reduction of GHG emissions from ruminants has been achieved indirectly by enhancing production efficiencies within the U.S. Other than the typical production characteristics (e.g., milk production, beef production) other herd performance characteristics have changed (e.g., milk composition, age at first calving, and calving interval), which are essential for quantifying dairy's environmental footprint (Capper and Cady, 2012). Between 1944 and 2007, the carbon, water, and land footprints of milk produced in the U.S. decreased 63%, 90%, and 65% respectively (Capper et al., 2009). Similarly, milk production in 2017 required only 79% of the land, 70% of the water, and produced 80% of the GHG emissions of milk production in 2007 (Capper and Cady, 2020). The equivalent efficiency improvements in the U.S. beef production system have also resulted in notable environmental benefits such that beef production in 2007 required 88% of the water and 67% of the land required in 1977 (Capper, 2011). Over this same timescale, the carbon footprint of beef was reduced by 16.3% (Capper, 2011). These historical trends are encouraging because they show the relationships between emissions intensities and production efficiency; however, there is also a need to reduce absolute emissions globally. It is important to note that improved emissions intensities do not necessarily confer reductions in total emissions.

Collectively, both the historical trends and the future projections suggest that improving production systems to balance between environmental impacts and population demand for food will require significant scientific advances. With the consumption of animal-sourced foods increasing, so too do concerns about enhancing the human population's sustainability and food supply security. The increase in animal protein consumption is projected to negatively impact the environment, particularly through increased GHG emissions (Henchion et al., 2017). However, the advancements in animal production efficiency achieved over the past several decades suggest a path forward to simultaneously increase animal-source food availability while limiting environmental impacts and improving resource use efficiency.

Alternatives to cattle production

An extreme alternative solution to addressing the environmental challenges associated with animal agriculture is eliminating or reducing animal-source food consumption, which is frequently proposed as a solution within the scientific and public policy literature (Afshin et al., 2019). For example, Hayek et al. (2020) predicted that if the population were to switch to only plant-based diets by 2050, there would be a reduction of 332 to 547 $GtCO_2$. A decrease of this size would be equivalent to the depletion of 99 to 163% of annual CO_2 emissions, based on 2019 metrics, which would improve the chance of limiting warming to 1.5 C by 66% (Hayek et al., 2020). Although there are clear environmental benefits potentially realized from eliminating or reducing animal-sourced food consumption, human nutritional and socioeconomic ramifications must concurrently be assessed (White and Hall, 2017). Additionally, when analyzing the impact of animal-sourced foods on the American diet, animal-sourced foods are essential for minimum-cost nutritionally adequate diets (Chungchunlam et al., 2020). In 2016, the FAO found that approximately 18% of the global calories ingested were from animal-sourced foods and 34% of the worldwide protein consumption was from animal-sourced foods (FAO, 2018). In addition to protein, which both beef and dairy provide a substantial amount of, beef and dairy also provide several micronutrients that are difficult to obtain in the required amounts from plant-sourced foods such as; Vitamin A, Vitamin B12, Calcium, and iron (Mottet et al., 2017). It has been estimated that cattle-produced milk contributes 28.5% of the requirement of the U.S. population, 43% for Calcium, 26 to 46% for amino acids, and 75% for Vitamin A (Davis and White, 2020). Similarly, the U.S. beef supply provides 10.5% of EPA+DHA and 15.5% of Vitamin B12 of the U.S. population (Gleason and White, 2019). Considering the implications of dramatic dietary shifts on population health, food equity, and accessible nutrition is critical when considering strategies to improve the environmental impact of the food production system.

Another consequence not often considered when proposing to eliminate animal agriculture is the upcycling potential of ruminant animals. We often assume food production would be more sustainable if all plant calories fed to ruminants would be better utilized by human consumption (Layman, 2018). However, this assumption fails to consider the diet of ruminant animals, which is predominantly forage. In the U.S., for example, approximately 85% of the beef cattle diet comes from plant materials such as grasses and hay (Layman, 2018). These plant feedstuffs are not digestible by humans, but the ruminant has the unique ability to consume and digest these plant materials efficiently (Patel et al., 2017). Additionally, ruminants can consume waste products such as distillers grains, corn leaves, wheat middlings, to name a few (Bath, 2018). If ruminants did not consume these waste products, the associated biomass would likely be removed from the food production system. Cattle can take these co-products of human food production and produce approximately 1.0 g of protein from every 0.6 g of co-product consumed (Mottet et al., 2017). When planning food system strategies to enhance environmental efficiency, optimizing the efficiency of the recycling role of cattle is also critically important.

Strategies to enhance cattle production efficiency

An alternative to a drastic shift in human diets is to improve food production sustainability by enhancing the efficiency of agricultural processes (Horrigan et al., 2002). As a general rule, there are three ways to enhance efficiency: increasing the amount of food produced per unit time (Pretty et al., 2003); increasing the amount of food produced per unit resource input (Pretty et al., 2003); and reducing the number of animals required to produce a quantity of food (Herrero et al., 2013). Strategies such as deploying growth-enhancing technologies (Capper and Hayes, 2012); adapting feeding practices to increase rates of gain (Brown et al., 2006); and genetically selecting animals to increase the efficiency of animal production all contribute to improving the speed of animal product production (Boichard et al., 2015) (increasing food produced per unit time). Strategies such as precision ration formulation (Tylutki et al., 2002), ionophore use (Callaway et al., 2003), and management-intensive grazing can contribute to improving the resource-use efficiency of production systems (Dartt et al., 1999) (increasing food output per unit resource input). With the implementation of growth-enhancing technologies such as steroid implants, beta-agonist, and ionophores, beef efficiencies have improved over the years (Capper and Hayes, 2012). Additionally, the improvements in reproductive efficiencies and cross-breeding techniques have also improved the whole-system efficiency of U.S. beef production. It is estimated that in 2000 and 2019 total U.S. beef production was approximately equivalent, at 12.4 billion kgs (Hilton, 2019). In 2000 the U.S. beef cow inventory was just over 35 million head. The inventory was around 32 million in 2019 (USDA, NASS). The drop in the total number of cattle and the same output of beef shows a clear improvement in beef production efficiencies over the past 2 decades. Additional changes over this timeframe which have contributed to improved animal use efficiency include improving breeding rates (Terry et al., 2020); enhancing finishing weights/lifetime milk yields (VandeHaar and St-Pierre, 2006; White and Capper, 2013); and reducing death losses (Ott et al., 1995). Although it is nice to characterize the system in this reasonably straightforward manner, it is important to note that each strategy has system-level effects, strategies often couple be implemented under more than one efficiency enhancing approach, andthe three approaches to enhancing efficiency often interconnect.

When evaluating the opportunities to enhance whole-system efficiency moving forward, it can be challenging to identify the relative expected payout of different strategies. For example, it may be possible to shorten the finishing period of cattle by approximately 2 months, though generating an exact expected change would require knowledge of the biological limit for efficiency, which is unknown for any agricultural species. One area for which we do have a good understanding of a biological limit (or, in this case, more of a biological target), is reproduction. Biologically and economically, it appears ideal for beef and dairy cattle to produce one calf per cow per year (Radostits, 1983). Average reproductive rates in beef and dairy production systems currently fail to reach this optima, averaging 91.5% (Hilton, 2019) and 59.7% (CDCB), respectively. This suboptimal reproductive rate also inhibits genetically-induced changes in efficiency, and optimizing reproductive and genetic management simultaneously are estimated to have substantial payoff for improving system environmental impact and economic viability (White et al., 2014). As such, one critical target within the broader effort to improve beef and dairy cattle production sustainability is to enhance reproductive efficiency toward the target of one calf per cow per year.

BOVINE REPRODUCTION

The Need to Enhance Reproductive Efficiency

Infertility in cattle is broadly defined as the diminished or absent capacity to produce viable offspring. The incidence of infertility and can be very costly for both beef and dairy operations. Due to the broad nature of the term, infertility can be categorized into three general types: 1) cows that do not become pregnant during a specified breeding season; 2) cows that successfully become pregnant but fail to carry the calf to term; and 3) cows that become pregnant, but do so at an inopportune time (Davis and White, 2020). The current U.S. beef and dairy industries have prime examples of how these different types of infertility can present. For example, in the decades before 2000, it was apparent that increasing milk production of dairy cattle was correlated with a decrease in reproductive efficiency (Nebel and McGilliard, 1993). The data suggest a negative genetic correlation between the high milk production and reproductive success (Hermas et al., 1987; Pryce et al., 1999) which includes both cows failing to successfully become pregnant and cows which fail to carry the calf to term. Similarly, the average calving interval within the U.S. dairy industry has increased by 1.5 months from 1980 to 2000, which suggests the challenge of cows becoming pregnant at inopportune times (Oltenacu and Algers, 2005). However, more recent literature suggests that since the year 2000 the reproductive success has improved in dairy cattle over the years (Wolfenson and Roth, 2019). These results certainly indicate that improvements are being made, however, there is still more research to be done to improve the dairy cow’s reproductive efficiency. Within the beef industry, challenges with reproductive efficiency most commonly present with replacement heifers, due to the biological and subsequent economic challenges associated with meeting the nutrient and energy demands for growth and reproduction simultaneously.

There are several metrics used to determine which replacement heifers to select when attempting to enhance operation profitability. Typically, age at puberty is one factor which is important to consider when selecting replacement heifers due to its correlation with lifetime fertility and production (Gutierrez et al., 2014). Short and Bellows (1971) showed that heifers that achieved puberty earlier, were bred earlier, calved earlier, and weaned heavier calves. Heifers which were pubescent at a younger age also tend to maintain these superiorities (calving earlier, weaning heavier calves) throughout their reproductive lifetimes (Lesmeister et al., 1973). Ideally, heifers reach puberty around the age of 15 months. However, as many as 35% fail to reach puberty by that age, which can negatively affect operational efficiency through longer growth periods, delayed breeding, and late calving (Patterson et al., 1992). Despite the fact that the beef industry generally has much greater reproductive success rates when compared with the dairy industry, there is still considerable opportunity to improve. Before evaluating strategies to enhance reproductive efficiency, it is useful to review the different approaches to reproductive management and how they are currently employed within the cattle production industries in the U.S.

Reproductive management strategies-natural service

The vast majority of beef cow producers breed their animals using the natural service method; i.e., the usage of live bulls in their herd to breed cows. It is estimated that 95.7% of beef operations use this breeding method (NAHMS, 2009). The breeding season for natural service can be controlled based on when the bull is co-housed with cows and heifers. Industry practices for defining a breeding season though co-housing range from allowing the bull access for 1 to 2 months, all the way up to continuous cohabitation with cows and heifers. Despite several decades of Extension efforts describing the benefits of a defined breeding season (i.e., creating a close calving window; improving calf uniformity and age; and better management of calving difficulty), over 50% of the U.S. beef operations do not have a set breeding season (NAHMS, 2009). In contrast, the remaining operations will typically have 1 to 2 breeding seasons, with 34% of them having a single breeding season, generally in the spring months (NAHMS, 2009). Operational challenges which preclude more operations from creating a defined breeding season include: lack of bull housing facilities; pasture or feed resource limitations; labor limitations; among others (NAHMS, 2009). Despite the challenges associated with defining a breeding season, there are a number of advantages associated with natural service breeding, which include reduced labor hours, minimal estrus synchronization and breeding costs, and high convenience (Karisch, 2020). One major limitation of natural service breeding is the lack of opportunity to make concerted and precise genetic advancements (Baruselli et al., 2018), and because of this limitation numerous professionals, researchers, and consultants have recommended broader adoption of artificial insemination (AI) within the beef industry (Butcer, 2017; Baruselli et al., 2018). Indeed, the adoption of AI practices within the dairy industry over the past several decades has contributed substantially to the productivity gains achieved over this timescale (Doormaal and Kistemaker, 2011).

Reproductive management strategies-artificial insemination

Artificial insemination has contributed notably to genetic gains because it is a breeding technique that provides producers the ability to inseminate cows with semen from proven sires irrespective of where that sire is located, or when he lived (Baruselli et al., 2018). These semen doses are typically less expensive than housing a bull in a natural service breeding system, and are often from animals of considerably greater genetic merit. Despite these advantages, only 7.9% of the beef industry used this assisted reproductive technology as of 2009 (NAHMS, 2009).

As might be expected, the use of AI on larger herds is greater than on smaller herds where the economy of scale is less pronounced (NAHMS, 2009). The beef industry has largely considered AI for breeding heifers (12.4%) more thoroughly than for breeding cows (4.1%). The reason for this difference in willingness to use AI is likely for a variety of reasons. First, producers do not want to injure their smaller heifers with the larger bulls (Dahlen, 2014). Second, bulls selected specifically for calving ease can be used on heifers to reduce risk of dystocia (Funnell and Hilton, 2016). Finally, producers see value in using the highest quality genetics with their higher quality females (i.e., heifers; Baruselli et al., 2018). Reasons provided by producers for why they do not utilize AI include labor and time (38.4%), financial reasons (19%), and that the process is too onerous or complicated (16.6%; NAHMS, 2009). In contrast to these reported challenges, experimental research suggests that the usage of AI increases the weaning weight of offspring, and narrows the calving window to allow for more calves to be born earlier (Steichen, 2013). Data from the dairy industry shows dramatic improvements in herd efficiency associated with the adoption of AI and genetic improvement because the present production system requires only half the number of dairy cows required to produce 1 billion kg of milk in 1960 (Capper et al., 2009). One critical challenge of AI revealed within the dairy industry is that of estrus detection. Success rates of AI breeding vary considerably, and are highly dependent on estrus detection methods (Nebel and Jobst, 1998).

Estrus synchronization

Estrus synchronization is the process of manipulating the estrous cycle (Odde, 1990), and is one strategy used in conjunction with AI to improve efficiency and accuracy of estrus detection. Estrus synchronization technologies provide the ability to manipulate the cow's natural estrus cycle and have been shown to shorten the calving window, reduce labor costs, and aid in culling decisions (Holm et al., 2008; De Vries et al., 2010). To manipulate the estrous cycle, several reproductive hormones are utilized, including: progesterone (P4), gonadotropin-releasing hormone (GnRH), and prostaglandin F2alpha (PGF2α). Estradiol (E2) is also used in some situations (Day et al., 2000). However, it is not legalized for use in the U.S. for production purposes; therefore, it will not be discussed in this review as an estrus synchronization technology. Additionally, follicle-stimulating hormone (FSH) is primarily utilized to induce multiple ovulations at one time (superovulation) to flush that animal and transfer her embryos into recipient cows to multiply her genetics (Monniaux et al., 1983). The practice of flushing is not widely used in typical production settings (NAHMS, 2009) and is also outside the scope of this review.

There are several protocols available to producers to more effectively synchronize the estrous cycles of their herd. These protocols are outlined by the Beef Reproduction Task Force (BRTF) and are tailored to different situations (i.e., labor convenience, heifers vs. cows, cost management). The typical synchronization procedures for TAI include; Ov-synch and Co-synch + CIDR. The Ov-synch protocol begins by administering 1 administration of GnRH, followed by an administration of PGF2α 7 days later, then a second dose of GnRH 2 days after PGF2α, and then TAI 12 – 24 h later (Melendez et al., 2006). The Co-synch + CIDR protocol is similar as it involves 1 administration of GnRH and an insertion of a controlled internal drug release (CIDR) object that releases progesterone into the vagina of the cow, followed 7 d later when the CIDR is removed and PGF2α is administered, followed 60 to 66 h later when a final GnRH dose is administered at the time of AI (BRFT, 2020). For heifers, a long term CIDR protocol has been shown to be beneficial for the induction of cyclicity of those animals that are prepubertal (Patterson et al., 1990). The long term usage of the CIDR (14-16d) is beneficial to successful presynchronization of heifers (Leitman et al., 2009). Ideally, these protocols will cause a large percentage of the herd to enter standing estrus within a relatively narrow timespan, allowing for more targeted breeding to maximize AI pregnancy percentages (Odde, 1990). However, for these protocols to work to their maximum potential, absolute conformity to the timeline and protocol steps is crucial. A more in-depth discussion about the naturally occurring and exogenous sex hormones helps highlight how estrus synchronization protocols can modulate the naturally occurring estrus cycle.

Progesterone

Progesterone is a key steroid hormone not only essential within the estrous cycle endocrinology but also to the growth and health of the fetus. Progesterone is produced by the corpus luteum (CL) of the cycling mammal (Niswender et al., 2000). However, in the pregnant cow, progesterone is also produced by the placenta during pregnancy (Donaldson et al., 1970; Henricks et al., 1972; Senger, 2005). Receptors for progesterone are primarily found in the endometrium and stroma while the animal is cycling (Maslar et al., 1986). Additionally, these receptors are located in the myometrium during pregnancy (Spencer et al., 2004). The highest progesterone concentration is achieved during the luteal phase of the estrous cycle or during pregnancy (Senger, 2005). These progesterone levels typically rise to around 6.0 to 9.9 ng/ml in blood plasma during early pregnancy (day 12) (Donaldson et al., 1970; Henricks et al., 1972).

Progesterone has to be suppressed or depleted by luteolysis for the female to enter into estrus, and estrus will only occur if no exogenous progesterone is supplied (Odde, 1990). If females have a regressing CL, exogenous progesterone can be supplemented to delay presentation of estrus. There are two primary forms of progesterone supplementation used in estrus synchronization: melengestrol acetate (MGA) and a controlled internal drug release (CIDR). Melengestrol acetate is typically supplied as a feed additive as an orally active progestin steroid (17-acetoxy-6-methyl-16-methyl-enepregna-4,6-diene 3,20-dione). Melengestrol acetate was developed to aid in the maintenance of pregnancy, delay the onset of estrus, and promote endometrial proliferation (Duncan et al., 1964). As a feed additive, MGA is typically a pelleted product and is used to synchronize estrus in bovine females (Brown et al., 1988; Kojima et al., 2000). The feed additive MGA is fed at a rate of 0.5 mg/d as suggested by Zoetis (Zoetis, Parsippany, New Jersey). The majority of MGA is fed to heifers in feedlots to suppress estrus in order to improve growth (Patterson et al., 1989). There are some challenges associated with MGA feeding, mostly when the animal does not consume the necessary amount to inhibit estrus (Lamb, 2013). Controlled internal drug release devices (CIDR) are more reliable in that regard because they contain exogenous progestin steroids that will pass through the vagina and into the bloodstream at a controlled and constant rate (FDA, 2019). The CIDR marketed in the U.S. is a t-shaped device covered with 1.38 g of progesterone (Zoetis, 2015). When the CIDR is inserted into the cow’s vagina, the blood plasma concentration of progesterone rises to between 4 and 6 ng/ml within hours of the initial administration (Dahlen et al., 2014). Typically, a CIDR is left in for 7d, but some protocols leave the CIDR in for up to 14 d (BRTF, 2020). Once the CIDR is removed, the plasma concentrations of progesterone falls within 24 hours to less than 1 ng/ml if a CL is not present (Dahlen et al., 2014). In this way, placement and removal of a CIDR is often an important step in estrous synchronization.

Prostaglandin

There are several prostaglandin hormones present in the mammal, which contribute to a variety of different functions. The prostaglandin hormones are fatty acid bases which exhibit hormone-like actions (Senger, 2005). For reproduction, prostaglandin F2alpha (PGF2α) is of primary importance. The uterus secretes PGF2α in a pulsatile fashion with approximately five one-hour long pulses per day (McCracken et al., 1984). The receptors for PGF2α are on the luteal cells of the CL (Arosh et al., 2004). Luteolysis occurs very quickly because PGF2α acts on the CL and effectively lyses the CL to begin atresia and regression (Odde, 1990). Without the CL present, the progesterone concentration and production will decrease (Silvia et al., 1991). Following luteolysis, there is no longer negative feedback on the growing dominant follicle and the lack of negative feedback allows for the follicle to increase estradiol production (Rathbone et al., 2001). The increase in estradiol eventually has a stimulatory effect on the hypothalamic-pituitary axis and causes an increase in the gonadotropin releasing hormone production (GnRH). The resultant dramatic rise in GnRH concentration then stimulates production of luteinizing hormone (LH), and induces ovulation of the dominant follicle and subsequent formation of a new CL (Rathbone et al., 2001)

As mentioned, PGF2α causes luteolysis, effectively removes the CL, and by association decreases progesterone production. For PGF2α to be effective, however, there has to be a functional CL present. Assuming d 0 is the day of ovulation, if PGF2α is administered anywhere between 5 and 16 of the bovine estrous cycle, luteolysis will occur (Rathbone et al., 2001). Independent of pharmaceutical intervention, luteolysis timing can be affected by factors such as age, breed, and body condition (Rathbone et al., 2001). After luteolysis, ovulation is expected occur, and a standing estrus should be observed. As discussed previously, detection of this standing estrus indicates the ideal time for AI or natural service breeding.

Gonadotropin-releasing hormone

Gonadotrophin-releasing hormone (GnRH) is a decapeptide hormone released by the mammal's hypothalamus (Thatcher et al., 1993). The hypothalamus releases GnRH in a pulsatile manner (Skinner et al., 1997), and after release it travels to the anterior pituitary. At the anterior pituitary, GnRH will stimulate the release of the gonadotropin hormones: follicle-stimulating hormone (FSH) and luteinizing hormone (LH; Naor et al., 2000). Follicle growth is stimulated by FSH and will peak when the dominant follicle reaches approximately 5 mm in diameter (Ginther et al., 2003). Peters et al. (1985) found that repeated small dose injections of GnRH over 2 d in dairy cattle resulted in enhanced follicle growth. After the dominant follicle reaches 5 mm in diameter, there is a more substantial inhibitory effect on FSH that will decrease the serum concentration (Ginther et al., 2003). Once the FSH begins to decrease, estradiol concentration increases due to the larger size of the dominant follicle (Wiltbank et al., 2002). When estradiol increases, there is a positive effect on GnRH later in the estrous cycle (d 18-20) and this shift in regulation will induce an LH surge (Wiltbank et al., 2002). The LH surge subsequently induces ovulation and the growth of a new CL for the next estrous cycle or pregnancy. Predominantly, GnRH is utilized in estrus synchronization protocols to induce ovulation (Islam, 2011). When exogenous GnRH is provided, it will allow for the dominant follicle to ovulate if it is larger than 10 to 15 mm, thus beginning a new follicular wave (Lucy et al., 1992).

Days to ovulation postpartum

The reestablishment of cyclicity following calving relies on normal gonadotropin cyclical patterns (Wettemann, 1980). Post parturition, cattle experience a period of sexual quiescence (anestrus; Short et al., 1990). For a cow/calf producer to achieve the aforementioned target of one cow per calf per year, the cow must resume fertile cyclicity by 90 days post-calving. For successful cyclicity resumption, the cow must undergo uterine involution where waves of vasoconstriction and contractions occur to expel the fetal membranes and shrink the uterus size (Kiracofe, 1980). Uterine involution appears to be an important factor in contributing to the resumption of a fertile reproductive cycle (Bellows and Short, 1994). However, uterine repair can still have a negative effect on fertilization and pregnancy in the first 20 days postpartum (Short et al., 1990). The estrous cycle immediately following parturition is often a silent estrus, meaning that the animal does not show the typical behavior during estrus despite undergoing the corresponding physiological changes (Perry et al., 1991). Additionally, some animals will show a short estrous cycle or a "short-cycle" between the first and second estrous cycle following parturition. The short cycle is an estrous cycle that is less than 17 d and is often 7 to 10 d (Odde et al., 1980). The short cycle occurs because premature uterine secretion of PGF2α lyses the CL formed, therefore only allowing progesterone to be supplied for a short time (Perry et al., 1991; Zollers et al., 1993). Ramirez-Godinez et al. (1981) claim that the short cycle is necessary to prime the animal for a normal estrous cycle to follow afterward. If the animal is at an acceptable energy balance, the GnRH axis will begin after this short-cycle, returning the cow to a normal state of fertility.

Oocyte growth/selection and ovulation

During the estrous cycle, there is a saturation growth pattern of follicular development as different follicular waves progress. Assuming a follicular wave is just beginning due to atresia of the previous wave or because ovulation was accomplished, the first stage of follicular growth is mostly independent of gonadotropin hormones (Gong et al., 1996). There is still little known about how a pre-antral follicle is selected to grow; however, the Foxo3 transcription factor is inhibited by Kit-ligand and allows the oocyte to grow (John et al., 2009). Following this stage, the follicle continues to grow and eventually becomes a secondary follicle (Fortune, 2003). The secondary stage is when the zona pellucida is formed (Chiquoine, 1960), and granulosa cells develop into several layers (Alam and Miyano, 2020). Subsequently, the oocyte's antrum develops and grows as the oocyte enters the tertiary stage (Latham et al., 2004). The tertiary follicle continues to grow and eventually reaches approximately 4 mm. At that stage of growth, the follicle is dependent on gonadotropin hormones, particularly follicle-stimulated hormone (FSH) (Latham et al., 2004). When the tertiary follicle grows, so too does the oocyte's antrum, and the follicle is considered a Graafian follicle. Typically, several follicles reach the Graafian stage, however, several will grow and regress throughout the estrus cycle (Scaramuzzi et al., 1980). Typically, all but one follicle will become atretic by the time of ovulation due to inhibin inducing a negative feedback on FSH production (Mihm et al., 1999). The selected dominant follicle inhibits new follicles' recruitment to the next follicular wave (Lucy et al., 1992). Assuming that the progesterone level is low, the large dominant follicle is continually producing estradiol, which at this stage in the cycle will improve the release of GnRH and enhance GnRH sensitivity within the pituitary gland (Kesner et al., 1981). The increase in GnRH release and sensitivity stimulate an increased luteinizing hormone (LH) pulse frequency and subsequently induce the dominant follicle's ovulation (Kesner et al., 1981).

Fertilization/embryonic development

The details of fertilization are partially dependent on breeding method. For example, the site of sperm deposition during natural service breeding is the vagina whereas sperm deposition from correctly performed artificial insemination occurs within the uterine body (Ortiz et al., 2019; Dalton et al., 2021). The sperm must travel from the appropriate deposition site up the ovulation side uterine horn and oviduct. The sperm then meets the oocyte at the junction of the ampulla and isthmus of the oviduct. Along the journey to the oocyte, the sperm undergo capacitation and increase their hyperactivity (Visconti and Kopf, 1998). Once the sperm reaches the oocyte, the sperm will bind to the oocyte's zona pellucida to induce fertilization (Brackett et al., 1980). Post fertilization, the pronuclei of the sperm cell and oocyte cell fuse and cause the first mitotic division (Yanagimachi, 1978). From there, the embryo will undergo many mitotic divisions to become a 4, 8, 16-cell embryo, and so on. When the cells multiply, there is a level of compaction still inside the zona pellucida; eventually, a cavity forms to create a “blastocyst” embryo (Eyestone and First, 1989). The cavity of the blastocyst begins to separate the trophectoderm from the inner cell mass. The trophectoderm is the perimeter lining of the embryo's cavity and will eventually develop into the conceptus once the embryo exits the zona pellucida (Betteridge and Fléchon, 1988). The inner cell mass will ultimately differentiate into the hypoblast and epiblast. The hypoblast then develops into the yolk sac and the endoderm (Zernicka-Goetz et al., 2009). The epiblast will develop into the mesoderm and ectoderm (Tam and Zhou, 1996). All of which are essential structures necessary for the fetus to develop.

Embryo elongation and implantation

After the embryo reaches the blastocyst stage, the embryo will hatch from the zona pellucida and grow into a tubular form (Hue et al., 2012). This tubular form is termed the conceptus. The conceptus will begin elongation and double in length every day between d 9 to 16 when day of breeding is considered d 0 (Berg et al., 2010). Immediately following elongation the conceptus begins to attach to the epithelium of the uterus. It is crucial that this elongating conceptus produce a high amount of interferon tau, because interferon tau is considered the bovine maternal recognition of pregnancy and prevents the release of PGF2α (Thatcher et al., 1984). Due to the inhibition of PGF2α, luteolysis is prevented and the CL is allowed to survive and continue to produce progesterone to service the pregnancy (Bazer et al., 1996). The phenomenon of successful reproduction is still not entirely understood and certainly warrants further investigation to continually improve the efficiency of cattle operations.

The State of Knowledge and Gaps in Understanding of Reproductive Management

In cattle, the primary drivers of reproductive efficiency include; days to ovulation, submission rate, conception rate, and calving rate (Sanchez et al., 2018). A low days to ovulation postpartum is crucial for the animal to meet the one calf per cow per year goal. The submission rate, particularly in AI breeding situations, is indicative of the proportion of animals that exhibit a standing heat or are breeding eligible. Conception rate is indicative of the proportion of animals that were confirmed pregnant out of all animals that were bred. Lastly calving rate is the proportion of cows that successfully calved to term out of the animals that were determined to be pregnant. Reese et al. (2020) calculated that about 28.4% of total embryonic loss in beef cattle occurs by d 7, 3.9% during d 7 to 16, and 15.6% during d 16 to 32. Wiltbank et al. (2016) calculated that about 20-50% of total embryonic loss in dairy cattle occurs by d 7, 3.9% during d 7 to 16, and 15.6% during d16 to 32. The early embryonic loss represents the major reproductive challenge that should be addressed by the cattle industries. There is research to say that a greater progesterone concentration (Inskeep, 2004), larger follicle size (Perry et al., 2005), and greater gonadotropin concentration (Silke et al., 2002) can aid in reducing embryonic loss in cattle; however, strategies to attain these biological endpoints are not always clear.

Although extensive research has been conducted to reduce embryonic loss in cattle, robust management strategies to achieve these critical biological endpoints that appear essential in preventing early embryonic loss are limited. In dairy cattle particularly, further research needs to be conducted to evaluate how reaching these biological targets can help address the negative impact of the genetic correlation between high productivity and reproductive failure. A variety of management factors have been identified to increase reproductive performance including: estrus synchronization (Xu and Burton, 1999), breeding season selection (Wilson and Lacy, 2006), cattle genetics (Weigel, 2006), among others. However, many of these solutions are not necessarily translatable across different operation types and may require substantial changes in management style to adopt. As such, there is a need to evaluate how more flexible, lower-cost management strategies could be leveraged to improve progesterone and gonadotropin concentrations, leading to large follicles and improved conception rates.

One such approach is that of nutritional management. Dietary concentration of specific nutrients like protein (Butler, 1998) and fat (Grummer and Carroll, 1991) have been shown to impact reproductive outcomes. Similarly, an animals body condition score and plane of nutrition are strongly associated with reproductive success (D'Occhio et al., 2019). Although these linkages between nutrition and reproduction are well-established, the feasibility and details of strategies to leverage nutritional management to enhance reproductive efficiency warrant further study.

INFLUENCE OF NUTRITION ON REPRODUCTION

Microbes contribution to ruminant metabolism

The rumen of the cow is unique when compared to monogastric animals. The rumen contain billions of microbes which include bacteria, fungi, protozoa (Huws et al., 2018). Among the microbes present are different subspecies that have differing functions in digestion (Stewart et al., 2018). The overall amount and proportions of these different types of microbes is dependent on the ruminant’s diet (Ellison et al., 2017). The end products of these microbes digestion include; volatile fatty acids (VFA’s) (Dijkstra, 1994), ammonia (Chen and Russell, 1989), and gases (Wallace et al., 2015). The gases are the primary sources of waste energy and include the GHG’s mentioned previously (Wallace et al., 2015). Ammonia is a product of protein degradation by the microbes and it utilized by the microbes for protein synthesis (Slyter et al., 1979). The rumen microbes are essential for fiber degradation but can also be digested by the animal and utilized for protein metabolism (Dijkstra et al., 1998). However, a high level of ammonia can lead to ammonia toxicity and have detrimental effects on the animal (Bartley et al., 1981). The major end products of fermentation are the VFA, which include; propionate, acetate, and butyrate (Leng and Brett, 1966). Propionate is beneficial to energy supplies because it can undergo gluconeogenesis and contributes to about 60% of the ruminants glucose supply (Wiltrout and Satter, 1972). Propionate is the primary VFA end product from rapidly fermentable cereal grains (Ametaj et al., 2010), thus diets greater in rapidly fermentable carbohydrate are thought to push the animal toward utilization of glucose as a major energy source. Despite the biological importance of propionate as a glucose precursor, acetate is the VFA produced in the greatest quantities within the rumen. Acetate is beneficial for milk fat synthesis because it provides the necessary acetyl-CoA and half of the reducing equivalents necessary for lipogenesis (Bauman et al., 1970). Both acetate and butyrate concentrations or molar proportions tend to be increased on diets that are high in fiber, particularly forage (Sutton et al., 2003). Butyrate can be metabolized by the liver for the production of ketone bodies. These ketone bodies are beneficial as an energy source, particularly when the animal is in a negative energy balance because ketone bodies can also be used for fatty acid synthesis, and because butyrate can be a substitute for acetyl-CoA in lipogenesis of the adipose tissue (Ladeira et al., 2016). The importance of these end products of microbial digestion to the ruminant's health and production are an important topic in present day research, however, their influence on reproduction is not well studied.

Body condition and its relation to reproduction

Body condition scoring is a method to evaluate the nutritional status of the animal by estimating body fat composition (Eversole, 2009). Acceptable body condition and positive energy balance are crucial for reproductive success (Markusfeld et al., 1997). There are differences in the BCS scale between beef and dairy cows, with beef cows on a scale of 1 to 9 and dairy cows with a scale of 1 to 5 with the lowest number indicating emaciated and the highest indicating a very high degree of obesity (Richards et al., 1986; Roche, 2006). Beef cows are recommended to be at a BCS of 5 to 7 at calving (Eversole, 2009). Dairy cows are recommended to be at a BCS of 3 to 3.5 at calving to minimize the time between calving and the next fertile cycle (Allen, 2011). The resumption of cyclicity postpartum is critical for cows to conceive and calve in a timely manner. As mentioned previously, proper body condition and nutrition are fundamental for a timely resumption of cyclicity in bovine females (Hess et al., 2005). This is particularly true for the first-calf heifers, which are typically in the lowest energy balance due to the energy demands of milking, resumption of cyclicity, and the fact that they are continuing to grow. Morrison et al. (1999) showed that changes in the LW and BCS in mature cows at 90 d pre calving have less importance on the resumption of cyclicity timeline than BCS at calving. For pregnancy rates, Rae et al. (1993) found that beef cows with a BCS less than or equal to 4 had a pregnancy rate of 59%, while cows that had a BCS of greater than or equal to 5 had a pregnancy rate of 90%. Additionally, Ayres et al. (2014) found an increased percentage of first AI pregnancy rates when cattle were at their highest BCS at calving. Weik et al. (2020)

found a positive linear relationship between BCS pre-calving, BCS pre-breeding, and first AI pregnancy rate, indicating that an increase in BCS between calving and breeding will result in a greater pregnancy rate. The increase in BCS is indicative that the animals were on a positive plane of nutrition, meaning they were in a positive energy balance. In herds where cows and heifers are on a negative plane of nutrition, a lower number of animals return to estrus early in the breeding season (Rakestraw et al., 1986). Clearly, the existing literature supports a strong association between optimal body fat reserves and positive reproductive outcomes. Although this association could be solely controlled by energetics (e.g., the animal has sufficient energy to support gestation and other necessary maintenance or growth processes), there are likely additional mechanisms which support the association between appropriate body condition and reproduction.

Metabolic hormones

The influence that body condition can have on reproduction is well understood, therefore, in order to progress our understanding of how nutrition affects reproduction, the logical next step is the investigation of the roles that metabolic hormones can play in reproductive success. Plausible mechanisms linking nutrition and reproductive outcomes can be found in the overlap of nutritional and reproductive endocrinology. Hormones such as insulin-like growth factor 1 (IGF-1), growth hormone (GH), and leptin are involved in both nutritional and reproductive endocrine cascades. As such, these provide mechanisms whereby nutrition may influence reproduction.

IGF-1

Within the bovine, the liver is the major site of IGF-1 production (D’Occhio et al., 2019). The many functions of IGF-1 include cellular differentiation and proliferation in reproductive tissues (Keller et al., 1998), stimulating ovarian follicle production and quality (Ginther et al., 2002), and improving gonadotropin hormone function by enhancing gonadotropin receptor action (Scaramuzzi et al., 2006). In particular, IGF-1 has been found to stimulate proliferation and mitogenesis in granulosa cells (Spicer et al., 1993) and improve FSH responses to steroidogenesis in granulosa cells (Zhou et al., 2013).

Nutrition has clear effects on IGF-1 production which may have flow-through impacts on reproductive efficiency. In an extreme example, fatty liver in the dairy cow is a fairly common condition where extreme negative energy balance causes a cow to enter a state of ketosis (Grummer, 2008). During ketosis, the body breaks down fat tissue at a rate which exceeds the metabolic capacity within the liver and fat tissue accumulates in the liver. In association with the phenomenon, production of IGF-1 from the liver is decreased (Rukkwamsuk et al., 1999). Insulin enhances lipogenesis and hinders ketogenesis in the liver (Brockman, 1978; Brockman, 1979). Although there are no known data associating NEFA concentration and production of IGF-1, an indirect relationship has been found between the two (Konigsson et al., 2008). Likely, this is due to the negative energy balance and lower insulin concentration of the animal causing an increase in NEFA concentrations from the metabolic response to mobilize fat to supply energy (Drackley, 1999). Whereas, increased IGF-1 concentration is associated with an increase in insulin concentration and a positive energy balance in cattle (Fenwick et al., 2008).

GH

Growth hormone (GH) is released from the anterior pituitary and controls growth and lactation in cattle. Primarily, GH improves nutrient partitioning to aid in tissue development and milk production by suppressing insulin action (Etherton and Bauman, 1998). Mainly, this action is prevalent postpartum due to the onset of milk production and negative energy balance. There is research to suggest that GH can stimulate gametogenesis and steroidogenesis at the ovary (Hull and Harvey, 2001). However, there is difficulty studying GH in vivo due to the onset of IGF-1 release caused by GH and its known stimulation of gametogenesis and steroidogenesis on the ovary (Adashi et al., 1985). Although, there is difficulty determining the individual effects of GH on reproduction, there is a positive relationship between androgens and GH release, which may be responsible for puberty induced GH production and puberty induced growth rate (Awoniyi et al., 1999).

Leptin

Leptin is predominantly secreted by adipose tissue and acts on the hypothalamus to regulate metabolism and fat deposition (Friedman and Halaas, 1998). It has been proposed that leptin may signal reproductive tissues whether or not there is sufficient energy for proper reproductive function (Tataranni et al., 1997). As such, there is a potential role for leptin in governing mammal ovarian function to facilitate the resumption of cyclicity and subsequent embryo development. To further illustrate this linkage, there are leptin receptors on ovarian cells (Agarwal et al., 1999), oocytes (Ryan et al., 2002), cumulus cells (Cioffi et al., 1997), and embryos of mammals (Kawamura et al., 2002). Despite the presence of those receptors, there is some controversy about leptin’s effects on reproduction within the scientific literature. Some research has reported that LH can be stimulated by leptin (Agarwal et al., 1999). Other studies have found that leptin has an inhibitory effect on LH (Spicer and Francisco, 1997). Further work is needed to better understand how leptin can function as an intermediate between nutritional management and reproductive processes.

Collectively, metabolic hormones overlap with reproductive signaling cascades, influencing important factors like ovarian cell function, embryo development, and tissue hormone sensitivity. Although our understanding of IGF-1, insulin, and leptin is reasonably confirmed within the existing literature, we do not possess solid evidence of how feeding specific diets contributes to controlling of these hormones or subsequent reproductive processes. Within the literature, there is some targeted research focusing on the associations between fat or protein supplementation and reproduction, which is not always consistent (Bottger et al., 2002; Law et al., 2009). Going forward, it is necessary that research is conducted to fine tune the ideal dietary composition to more efficiently leverage dietary management as a strategy to optimize reproductive success. Reviewing the specific reproductive responses to individual nutrients which have been well studied provides context for defining future directions for targeted follow-up work.

Fat supplementation and reproduction

The effects of fat supplementation on reproductive outcomes within the published literature have been mixed. Bottger et al. (2002) conducted an experiment where beef heifers were supplemented with either high linoleic or high oleic safflower seeds and reported no differences in serum concentrations of IGF-1, IGF-1 binding proteins, non-esterified fatty acids (NEFA), GH, glucose, or insulin. However, Williams and Stanko, (2000) reported that polyunsaturated plant oil supplementation increased insulin and GH concentrations in both dairy and beef cows. It has also been reported that the feeding of supplementary fat almost always increases plasma NEFA (Staples et al., 1998) and cholesterol concentration. Some reasons for the differences observed among these studies may be associated with the variety of oilseeds utilized and the variability of digestibility (Funston, 2004). Additionally, the other dietary ingredients could be a confounding factor in the results of the fat supplementation study. For example, Bellows et al. (2001) conducted 2 studies involving fat supplementation and reproductive outcomes in heifers. In the first study, there was an increase in pregnancy rates in the fat supplementation group. In the second study there was no difference in pregnancy rates. A primary dietary difference among these studies was that the second study had a greater amount of forage available. The authors concluded that in that study the forage supply and quality was sufficient to supply sufficient energy and nutrients for reproductive success (Bellows et al., 2001).

Williams and Stanko (2000) found that dietary fat inclusion increased progesterone concentrations and the overall lifespan of the corpus luteum (CL). The increase in progesterone may reflect increased availability of cholesterol, which is a necessary precursor of progesterone. As such, there appears to be a beneficial relationship between dietary fat inclusion and CL function, which may impact reproductive performance. Fat supplementation also improves dietary energy, increasing the size of the preovulatory follicle (Lucy et al., 1991), and subsequently improving estrogen production from the follicle (Beam and Butler, 1997). This is beneficial because a larger follicle has been shown to enhance the quality of the oocyte and the corpus luteum function (Vasconcelos et al., 2001). These linkages between fat feeding, CL function, and oocyte characteristics are likely driven both by energetics and by endocrine responses. Fat supplementation does provides energy to meet the requirements associated with reproduction (Grummer and Carroll, 1991). Fat supplementation also shows an endocrine response in the ruminant. In particular a low n-6:n-3 ratio of fatty acids fed to cattle has resulted in a significantly lower plasma progesterone concentration (Zachut et al., 2010). Although these mechanisms of fat’s influence on reproductive outcomes have been identified, more specific research on the relative trade-offs among these modes of action is needed to better elucidate the relative importance of fat as an energy source versus fatty acids as signaling molecules. Furthermore, these studies have not clearly differentiated fat itself from improved energy balance, or evaluated how fat content in the diet may interact with other nutrients to impact reproductive outcomes. Future study of these relationships may be essential to designing feeding strategies to optimize reproductive success.

Protein supplementation and reproduction

Similar to fat supplementation, there are discrepancies in the findings of studies investigating the association between dietary protein content and reproductive performance (Law et al., 2009). In one study, when Holstein cows and heifers were fed a 13% or 20% CP diet, there were no significant differences in pregnancy rates. However, McCormick et al. (1999) showed that Holstein cows fed a 23.1% CP diet exhibited a 22% lower pregnancy rate when compared to the other group fed a 17.7% CP diet. Diets higher in CP are more likely to produce higher urea nitrogen concentrations (Claypool et al., 1980). High urea production occurs most often when the cow is in a negative energy balance (Laven et al., 2007). The increase in urea nitrogen can increase risk of ammonia toxicity (Patra, 2015), which is also associated with increased incidence of early embryonic loss and adverse effects on oocyte gametes (Leroy et al., 2008). Additionally, urea may have adverse effects on progesterone action on the uterus, creating a suboptimal environment for embryo implantation (Butler, 2000). Urea has also been shown to increase the secretion of PGF2α, which also may harm embryo viability (Butler, 1998). Blanchard et al. (1990) found that embryo quality and development were reduced in lactating cows fed excess rumen degradable protein. However, Gath et al. (1999) found no significant difference in embryo transfer and superovulation experiments when beef heifers were fed excess

CP. In addition to the lower pregnancy parameters, Carroll et al. (1988) also found that when postpartum cows were fed a 20% or 15% CP diet, there was a significantly longer anestrous period for cows fed the 20% CP diet. The discrepancies among reproductive responses to protein supply within the rumen may be partially due to differences in basal diet, amino acid profile, degradability profile of protein, or other experimental differences between these studies. For example, a diet high in CP had a negative effect on embryo viability in lactating cows (Butler, 1998). However, in a beef cattle study there was no negative effect on embryo viability when fed a diet high in CP (Gath et al., 1999). Likely, these differences observed are from relative energy balance differences in the lactating dairy cow and beef heifers.

Much like with fat supplementation, the variable relationship between dietary protein supply and reproductive outcomes warrants further investigation. Amino acids have been well established in most protein synthesis tasks as substrate for protein synthesis but also a potent regulator of protein synthesis through the MTOR signaling pathway (Wang and Proud, 2006). As such, there are complex biological mechanisms through which amino acid supply may affect reproductive outcomes. The relative importance of total protein supply, amino acid balance, supply of individual amino acids, and the interaction of protein or amino acid with other nutrients becomes particularly challenging to study within the ruminant because of the ways that the rumen microflora modulate amino acid profiles (Liebe et al., 2018). As such, future work is required to better understand how different protein classifiers (e.g., CP, RUP, RDP, MP, MAA, etc.) impact reproduction, how they interact with other nutrient supplies, and how they serve both as substrate for and modulators of protein synthesis tasks associated with reproduction.

Reproductive Responses to Dietary Starch and Fiber

As mentioned previously, increased dietary starch intake is related to elevated propionate production. Propionate is a primary factor in gluconeogenesis in the ruminant, therefore an increased amount of starch contributes to whole-body glucose supplies (Reynolds, 2006). Glucose causes an increase in insulin and IGF-1 production, which have both been shown to improve follicular cell proliferation and steroidogenesis (Spicer and Echternkamp, 1995). Dyck et al. (2011) found that diets higher in starch resulted in a significantly lower days to reach a dominant follicle ≥ 10 mm, higher number of LH peaks, and a decreased days to ovulation postpartum. Additionally, Gong et al. (2002) found that greater starch intake resulted in a greater proportion of cows that ovulated before 50 days postpartum. Bedford et al. (2018) found that a propionate infusion in dairy heifers initially resulted in an increase in circulating propionate concentrations. However, these concentrations eventually dropped, signifying that the heifers shifted metabolically towards an increased conversion of propionate to glucose. Additionally, the propionate infusion group had elevated progesterone concentrations at 14 d post ovulation when compared to the control group which received a saline infusion (Bedford et al., 2018). These results suggest that a diet rich in starch could improve reproductive performance through propionate production and its downstream endocrinological responses.

There is little research that investigates the relationship between dietary fiber and reproductive performance in cattle. However, as mentioned previously, acetate and butyrate are the primary VFA's produces from the fermentation of fibrous feed ingredients (Kljak et al., 2017). Acetate can contribute to gluconeogenesis through its contribution of acetyl-Coa, however these contributions of acetyl-CoA are negligible when compared to propionate (Stocks and Allen, 2012). Additionally, butyrate can be converted to beta-hydroxybutyrate (BHB) by the rumen epithelium and by the liver (Holtenius and Holtenius, 1996) and is often viewed as a major energy source for the gut tissues. The relative concentration of circulating BHB has been linked to delayed timing of pregnancy in cattle and has been hypothesized as a potential predictor of reproductive performance (Walsh et al., 2007; Mulliniks et al., 2013). In addition to acetate and butyrate as a potential avenue for fiber to influence reproduction, fiber is beneficial for overall rumen health due to its affect on reducing metabolic disorders, rumen fill, and regulation of ruminal pH (Curtis et al., 1985; Bernard and McNeill, 1991). Metabolic disease is commonly linked with poor health and negative production outcomes, as such maintenance of health status and rumen health, specifically, may indirectly contribute to improved reproductive outcomes.

The State of Knowledge and Gaps in Understanding of Nutritional Modulation of Reproduction

The current literature suggests that there is an association between diet composition and reproductive efficiency, which is often supported by our current understanding of physiological processes involved in digestion, metabolism, and reproduction. There is still little known about the duration of dietary supplementation required to influence reproductive success, what types of feed, and the dietary inclusion of these feed supplements needed to improve reproduction. The scientific literature suggests variable impacts of protein and fat supplementation on reproduction, which warrants further investigation. Additionally, there more research is necessary to investigate how starch, fiber, and their relative VFA concentrations can influence reproduction. Past literature has highlighted in great detail how simple metrics like plane of nutrition and body condition can influence reproduction, however, the next step is to evaluate how aspects of the dietary composition can impact reproductive success in cattle.

Summary and Implications

Reproduction in cattle is a complex process with numerous factors required to work in harmony for success. There are widely accepted technologies that aid in cattle reproduction efficiencies (i.e., estrus synchronization, AI, embryo transfer). However, these technologies are not widely adopted, particularly in beef systems, and strategies to improve ease-of-use and efficacy of assisted reproductive technologies are essential to advance the efficiency of ruminant production systems.

Energy status is a primary driver of reproductive success. Simplistically, if cows are in a negative energy balance, their chances of reproductive success are low. However, several studies suggest that dietary intervention with supplements of specific composition may improve reproductive efficiencies beyond what would be explained by transitioning to a positive energy balance. Therefore, more research is required to outline the effects of specific deviations in dietary composition on reproductive success in cattle. This dissertation will investigate the impact that nutrition can have on reproduction. The utilization of diet as a means to improving reproductive success is theoretically a low-cost and low-input method that will work in harmony with the benefits of reproductive technologies. The overarching goal is that with improved nutrition intervention the utilization of these technologies will become more utilized by the producers.

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CHAPTER 3

Breeding animals to feed people: the many roles of animal reproduction in ensuring global food security

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Abstract

As the population grows and shifts demographically, the resulting increase in demand for beef and milk necessitates improvements in the sustainability of ruminant livestock production systems. Ruminant livestock contribute to ensuring global food security because they have the ability to up-cycle non-human-edible products into meat and milk products with notable nutritional value. However, ruminant livestock also pose a challenge to global food sustainability because they are resource-intensive to produce and contribute substantially to agricultural greenhouse gas emissions. As such, improving environmental impacts of ruminant livestock production globally is an essential goal. There are a number of strategies that can be employed to enhance sustainability of ruminant production systems; however, improving reproductive efficiency is among the more efficient, because an increase in reproductive success will reduce the number of cows needed to produce a target quantity of beef. This reduction in the cow herd size helps limit the number of unproductive animals retained in the herd, thereby reducing the environmental maintenance cost of livestock production. Additionally, proper application of reproductive technologies enables faster and more targeted advances in genetic gains, which can be leveraged to produce phenotypes that are resource-use-efficient and well-adapted to their production environment. Optimizing reproductive efficiency can be accomplished through improved genetic selection for fertility and fecundity; applying more effective use of assisted reproductive technologies; and coupling reproductive and nutritional management to optimize likelihood of reproductive success. Collectively, applying these approaches will be essential when working to ensure ruminant livestock's contribution to global food security.

Keywords: Food Security; Reproduction; Environmental Impact

Introduction

By 2050 the global population is expected to reach 9.7 billion, and estimates for the 2100 population suggest additional growth to 11 billion [1]. Not only will the population increase over the next several decades but the per capita incomes of people in developing countries are expected to triple from 2004 to 2030 [2]. With this higher level of income, families are expected to increase consumption of meat and milk [3]. In conjunction with this growth in population and incomes there is also an extension in life span occurring in the world today. It is estimated that the population of individuals aged 60 years or older will more than triple from now to 2050 [4]. Collectively, these changes in population demographics make it essential to produce more food. Indeed, it is estimated we will need to increase food production as a whole by at least 70% [5]. To meet this demand annual meat production alone will need to rise by over 200 million tonnes per year [6]. In addition to the need to produce more food, that food must be produced more sustainably [7]. In practice, this means achieving the desired increase in meat and milk production while using fewer animals, less water, and less land. Global pressure on limiting greenhouse gas emissions also suggests that we must limit emissions from livestock production as much as possible. There are a number of strategies that can be used to enhance the sustainability of cattle production systems, irrespective of the management framework used for production [8]. The USDA defines sustainable agriculture as: an integrated system of plant and animal production practices having a site-specific application that will over the long-term: 1) satisfy human food and fiber needs; 2) enhance environmental quality and natural resources upon which the agricultural economy depends; 3) make efficient use of nonrenewable resources; 4) sustains economic viability of farm operations; and 5) enhance quality of life.

One strategy to enhance sustainability of cattle production systems is optimizing reproductive performance of cattle [9]. Infertility in cows represents an inhibition to productivity and can be categorized into three types: 1) cows that do not become pregnant during the breeding period; 2) cows that do become pregnant but do not carry successfully to term; and 3) cows that do become pregnant but become pregnant late during the breeding season or during an inopportune time. Given the importance of reproduction in sustaining productive beef and dairy operations, it is imperative that producers have a successful reproductive program to maintain profits and continue production [10,11]. The purpose of this review is to better summarize how maintaining reproductive viability of cattle contributes to the environmental impacts of beef and dairy products, and to the challenge of feeding the growing global population.

Trends in Global Food Insecurity and Agricultural Environmental Impact

To understand the role livestock products play in global food security and agricultural environmental impact, it is useful to establish context for the contributions of livestock. According to the UNFAO [12], between 2015 and 2017, an average of 803 million people (approximately 10.5% of the global population) were undernourished. According to the same data source, 685 million people (2.5% of the global population) were severely food insecure. According to the Contributions of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [13], Electricity and Heat Production account for 25% of global emissions, other energy sources add another 10%. Industry accounts for 21%, transportation for 14%, and buildings for 6% of total global emissions. Agriculture is combined with forestry and all other land uses and accounts for 24% of total global emissions. The FAO [12] data suggest approximately 45% of agricultural emissions are attributed to enteric methane emissions and total emissions from manure management. Although not all emissions associated with manure can be directly attributed to ruminants, but nearly 40% of agricultural emissions are from enteric methane, which is directly associated with ruminant production. These data clearly demonstrate the need to minimize the global role of ruminant livestock in emitting greenhouse gases. According to the UNFAO [12], 2,769 km^3 of water is used for agriculture per year. This accounts for 69% of total annual global water withdrawal. Agriculture in Asia accounts for 2,069 km^3 (75%) of agricultural water use. In 2015 [12], 32% of the global land area was tree-covered areas, 14% was terrestrial barren land, 14% was grassland, 13% was herbaceous crops, 12% was shrub-covered areas, 7% was sparsely natural vegetated areas, and the remaining 7% included artificial surfaces, inland water bodies, mangroves, glaciers, and woody crops. Of all land, only 27% was associated with agricultural activities like cropping and grassland production. Although agriculture is commonly cited as a major contributor to global environmental impacts [14], it is useful to leverage quantitative information to better characterize the precise role of animal agriculture in generation of global environmental impacts and to identify critical targets for improvement.

In addition to understanding the role of agriculture within the global context of food security and environmental impact, it is useful to characterize the recent trends in food production and agricultural impacts. The UNFAO data [12] suggests that undernourishment has decreased by 11.8 million people per year from 2000 to 2016. Similarly, the proportion of people using safely managed sanitation has increased by 0.75% per year, the dietary energy adequacy of diets has increased by 0.41% per year and the per capita protein supply has increased by 0.30

kg/person/year. Over the 57 years between 1960 and 2017, the UNFAO data [12] suggests that availability of plant and animal products have increased by 3 kg/person/year and 0.36 kg/person/year, respectively. Over the same timescale, crop and grassland area has decreased by 0.0071 ha/person/year, agricultural water use has decreased by 2.5 cubic meters/person/year, and agricultural GHG have increased by 1.2 kg of CO_2 equivalents/person/year. The historical data suggest the agricultural system is providing more food from fewer resources.

There are several additional points that should be considered along with these data. First, substantial variation exists in the environmental impact of ruminant production systems globally. Taking global numbers or averages and assuming that they confer to specific regions or production systems is not appropriate. To better understand how agriculture, or more specifically animal agriculture, contribute to the environmental impact or food provision at regional or national levels, geographically specific analyses are required. Additionally, the net changes in environmental efficiency should be considered in the context of total environmental impact. The data from FAO [11] suggest that agricultural greenhouse gas emissions are still increasing. Targeted improvements in emissions reductions are a critical goal for livestock production systems over the next few decades.

Contributions of Beef and Dairy Cattle to Global Food Supplies

Based on summation of individual country production levels, global production of milk ranged between 3.4 to 3.6 million tonnes between 2013 and 2017 [12]. Similarly, global production of meat ranged from 0.34 to 0.35 million tonnes. We used the milk composition data from the USDA Food's List [15] and the average nutrient requirements from White and Hall [16] to estimate what proportion of humans' nutrient requirements in each country are met from dairy products.

The milk produced by cattle alone provides: 28.5% of the average country's requirement for protein; 43% of the average country's requirement for Ca; 26 to 46% of the average country's requirement for amino acids (AA); and 75% of the average country's requirement for Vitamin A. Values for the relative contribution of beef to domestic nutrient supplies are lower, but beef does provide a notable proportion of domestically available vitamin B12 (15.5%) and EPA+DHA (10.5%). These production statistics alone suggest that milk and meat are essential components of our global food supplies, making up socially, economically, and nutritiously important components of the human diet.

Previous reviews [17] and studies [16] have highlighted the importance of maintaining livestock as a part of future food production systems. Livestock provide innumerable important utilities for humans. Meat and milk constitute some of the most bioavailable sources of protein, amino acids [18], vitamins [19], and minerals [20] available for human consumption. With global income on the rise the global consumption of animal proteins has increased from 17g/person/day in 1960 to 27g/person/day in 2013 (Defining a land boundary for sustainable livestock consumption). Livestock in developed and developing countries constitute an important social resource [21]. Despite these benefits, there have been a number of recent publications highlighting negative environmental and human health effects of livestock [22–24]. Indeed, these articles highlight concerns within the literature regarding the carbon footprint of livestock products and the healthfulness of these products. One major contributor to the carbon footprint of livestock globally is the emission of enteric methane. However, the representation of methane emissions in most carbon footprinting approaches published do not confer with a new method for accounting short-lived climate pollutants [25]. That said, the Intergovernmental Panel on Climate Change (IPCC) has also increased the global warming potential of methane and the potential counterbalancing effects of these updates in our understanding of atmospheric science should be taken into account when characterizing the environmental impact of ruminant livestock. Similarly, new analyses are emerging regarding the negative health claims attributed to red meat [26] suggesting that continued consideration of the most-undated literature, within the context of existing literature, is needed when discussing how livestock products contribute to global food security. Continual discussion and re-assessment of these subjects within the scientific community is important to ensure we appropriately define the role of livestock products in feeding our growing global population.

Environmental Implications of Enhanced Reproduction

Over the period from 1944 to 2014, substantial improvements in the carbon, land, and water footprints of dairy production were accomplished in the United States [27], this was due, in part, to the adoption of artificial insemination, which allowed for more selective breeding to improve productivity. Indeed, in an assessment of environmental impacts on beef production systems, enhancing genetic gain of animals while concurrently improving reproductive and nutritional management was the most effective strategy for reducing environmental impact when compared with applying stand-alone improvements in reproductive and nutritional management [9]. The implicit linkage between breeding cattle and advancing genetic merit means that getting the cow bred, and bred to the correct bull, is among the most efficient ways to improve environmental impacts of livestock production. Independent of the link with genetic gain, reducing the calving window and improving conception rates also were more effective ways to improve environmental impact than adjusting nutritional management alone [9]. Studies on the role of cow productivity, fertility, and longevity and how these factors impact global warming potential [28] of dairy systems also found reduced fertility to be an important factor affecting environmental impact [28,29]. These findings highlight the importance of the common management goal of producing one calf per cow per year.

A challenge with the goal of producing one calf per cow per year is the implication that, once achieved, not further benefit can be realized from enhancing reproductive efficiency. Based on the difficulty encountered when attempting to achieve this goal, it seems unlikely that we will soon get to a situation where we have all cows globally producing at this level. Although developed ruminant production systems often result in >0.8 calves per cow per year, many developing countries have much lower reproductive success. The calving interval of top dairy producing countries ranges from 369 d (New Zealand) to 455 d (Argentina), suggesting even our most efficient producers struggle to reach the 365 d target [29]. A number of factors contribute to poor calving rates in cattle (fertility, nutrition, management, etc.); however, a major factor to consider when thinking about the relationship between reproduction and environmental impact is climate. Climate projections suggest not only an increase in mean temperature, but an increased incidence of variable and extreme weather [30]. The link between impaired fertility and hot or extreme climates is well established [31–33]. An essential goal of the next few decades must be identifying strategies to sustain fertility of cattle when exposed to extreme and variable temperatures during breeding and gestation.

To more quantitively demonstrate the relationship between environmental impacts and reproductive efficiency, we simulated the changes in environmental impact of a U.S. beef productions system when the calves born per cow per 365 d was varied from 0.5 (baseline) to 1 (theoretical optimal; Figure 3.1). The 0.5 baseline was selected not because it is representative of a US system, but to demonstrate the realistic extent of the response surface that could be expected globally from changes in reproductive efficiency. A doubling of reproductive efficiency, for those counties that could sustain such a change on account of having very poor reproductive efficiency currently, could lead to a 34% reduction in water use, a 44% reduction in land use and a 39% reduction in carbon footprint. However, it is important to note that most countries have much higher baseline calving rates and this likely over-estimates the expected benefit from enhancing reproduction. As an example, if we use 0.9 as a baseline and evaluate the relative change of improving to 1 calf per cow per year, the resulting environmental impact reductions reflect a unilateral 12.5% improvement (3.8 to 4.8 percentage unit change).

An important consideration when trying to characterize the environmental benefits of improving reproductive efficiency is the current environmental impact of the production system. If the above system had a carbon footprint of 15 kg CO_2e per kg beef, the resultant reduction from doubling reproductive efficiency would be 5.8 kg CO_2e per kg beef. However, if the system had a carbon footprint of 30 kg CO_2e per kg beef, the result of the same proportional change in reproductive efficiency would be an 11.6 kg CO_2e per kg beef improvement. If the benefit of optimizing reproductive efficiency is going to be more completely realized, we should target systems with very poor reproductive efficiency, very large environmental footprints, and large numbers of animals.

Trends in Cattle Reproduction and Strategies to Optimize Reproductive Efficiency

Decline in fertility of cattle

The trend for a decline in fertility of cattle worldwide [34] is a major concern, not only for the longevity and economic viability of the industries but also for the environmental impact of meat and milk production. Whether the cause of the decline in dairy cows is due to physiology, nutrition, genetics, etc. there is no question that over the past five decades there has been a decrease in fertility of dairy cows in concurrence with the rise in milk production [35]. A number of reasons have been hypothesized to explain the observed decline of reproductive efficiency in the dairy industry. The predominant belief is that single-trait selection for enhanced milk production has supported production of cattle with unique physiological adaptations that prioritize milk over other productive process, like reproduction (i.e. successful breeding and calving). This prioritization appears most significantly in the early postpartum period of the cow [36]. Another consequence of high milk production is elevated dry matter intake, which resulted in elevated blood flow to the liver, where a large portion of progesterone is metabolised [11]. This enhanced metabolism of progesterone is also thought to contribute to the decline in fertility over time.

There are also indirect effects of high milk production on loss of reproduction. High production often results in negative energy balance and subsequent mobilization of body fat reserves, resulting in impaired body condition. Poor body condition is also associated with impaired immune function [37]. A poor immune system during lactation leaves the cow more vulnerable to diseases like Leptospirosis [38], Bovine Viral Diarrhea Virus [39], Brucella [40], etc. which are known for causing abortions in cattle . In addition to these indirect immune effects, high milk production is also correlated with risk of mastitis, hypocalcaemia, and lameness, all of which have a negative effect on reproductive success [40].

As a result of these physiological shifts along with other less-well-documented changes, during the period from 1980 to 2000 calving intervals increased from less than 13 months to over 14.5 months based off a study involving 143 US Commercial herds [41]. During the same time interval, the number of inseminations per cow increased from 2 to 3.5 [41]. These changes occurred in conjunction with a net decline in pregnancy rates of first AI/ service by 0.5% per year in the range of years 1975 to 1997 [40,41].

Despite the clear phenotypic trend, discerning what proportion of the change in phenotype is due to indirect selection against reproductive traits is challenging. A major limitation in our understanding of the role of genetic selection in reproductive traits is the low heritability of reproductive traits. Several groups have reported heritabilities of close to 0 [42] for reproductive traits in purebred production systems. There is some disagreement in the literature, for example, one group reported that the estimated heritability rate for days open is 0.05 [43]. A separate study reported the parenthetical heritability rates for number of services (0.01), conception rate (0.03), service period (0.02), days in milk at first breeding (0.13), days open (0.04), and age at first calving (0.12) [42]. Although these heritabilities are modest/moderate, they suggest that there is a repeatable genetic component to reproductive success that could be selected against during single-trait selection for milk or selected for to combat the currently observed depressions in fertility.

Use of Assisted Reproductive Technologies

As the industry pushes for improved productivity in both beef and dairy cattle production, there is a need for more precise breeding to best match genetics of sires and dams to continue to increase productivity. Assisted reproductive technologies like; artificial insemination, embryo transfer, and in vitro embryo production all provide promising methods to continue to better match genetics of livestock for improved progeny. These technologies currently differ somewhat dramatically in their scalability, efficacy, and cost-effectiveness.

Artificial insemination is one of the more widely-used assisted reproductive technologies. Through adoption of artificial insemination, dramatic improvements in production of milk, in particular, have been realized world-wide. These technologies offer the opportunity to use bulls from across the globe and significantly drop the need for natural service bulls. For example, it has been reported that from 1993 to 2003 milk yield increased 3.7% in the UK and 3.4% in Canada. It is believed that 50% of this increase in milk production is due to the added genetic value from AI. [40,41,44]. Use of artificial insemination is also adopted quite widely in many developed beef production systems. Although particularly convenient for beef producers, the use of natural service bulls on an operation can actually be more costly to an operation than using AI. With AI there is merely the cost of semen, the liquid nitrogen filling of a semen tank, estrus synchronization exogenous hormones if chosen to time AI, and the AI technician if chosen to hire due to inexperience by the producer. With the natural service method there is the cost to feed bulls throughout the year, even during non breeding seasons. And with that bull there is always the risk of infertility, lameness, damage to fences/other property, and the risk of danger to farm workers. Artificial insemination also improves environmental impact of livestock production systems because the maintenance environmental cost of keeping bulls is diluted across a much larger number of progeny.

Although applied in some production settings, embryos produced by superovulation or in vitro fertilization are more commonly used in purebred and seedstock breeding operations [45]. These technologies help to better distribute high-quality dam genetics and when they become more cost effective and feasible for implementation in production settings, may have an additive effect to artificial insemination in terms of improving productivity of progeny.

Cost of Reproduction

As each individual cow in an operation does not become pregnant when the producer desires, the cost of production to an operation goes up. The reproductive performance on a dairy and beef operation is a large contributor to overall profitability of an operation. Using fall 2019 beef fed calf prices ($3.13/kg [46]) from the United States (US) and assuming the producer is selling these live feeder calves at 227 kg, a 1% increase in cows bred on an operation can save the producer $7.10/cow/ year (227 x 3.13 x 1%) [47]. Although this may sound small to an individual producer, when applying this to the total number of beef cows in the US (~41 million in 2019) [48] it can save the US $291,100,000. In the US dairy industry, it is estimated that infertility alone costs the industry $473 to $484 million [49].

Another aspect to consider is the 21 day cycle of the cow. Each missed breeding opportunity (ovulation) pushes the calving window back approximately 21 d. This delay costs the producer labor costs due to the increased calving window and the need to extend heat checking. If using a timed AI breeding method, this additional labor is in addition to the input costs mentioned earlier. The economic incentive to improve reproductive efficiency is clear, and perhaps partially explains why reproductive technologies are generally well-adopted.

Several additional economic factors should be considered when assessing the impact of reproductive efficiency including the populations of culled cows and replacement heifers retained; feed costs; non-reproductive labor costs; and veterinary expenses. All these factors are affected by the reproductive efficiency of an operation [50]. If the producer is able to get more of their cows bred earlier then as time goes on there should be more animals raised on that operation due to the shortening of the postpartum to breeding interval. Collectively, assessing the relationship between economics and reproductive efficiency is critical when considering strategies to provide an affordable and secure food supply.

Use of heterosis

Most of the above discussion on leveraging breeding technologies to enhance genetic gain leverages knowledge obtained from purebred herds. Use of heterosis is quite common in commercial beef cattle, and previous comparisons suggest crossbred calves had a weaning weight 6.4% greater than the purebreds [51]. This however cannot be generalized to all operations or breeds, but rather it provide an example of the benefits provided by heterosis. As such, crossbreeding, or targeted use of heterosis, may be an effective way to enhance the productivity of livestock. In the dairy industry it is more difficult to envision a crossbreeding system taking place due to the industry being Holstein dominated (≅90% of all cattle in the industry) [52]. However, in some European production systems beef and dairy products are procured from a single production system. In these mixed suckler-beef systems, leveraging technologies like sexed-semen can help to produce the best beef males to be raised as steers for beef and replacement dairy females from a common set of dairy-breed dams. This can better be accomplished by using genotyping technologies, which are constantly improving [53]. Genotyping technology enables the producer to better estimate which cows will produce females that will milk the best. Considering this knowledge they can use female sexed semen on their best genotyped cows to ideally retain those female genetics. Correspondingly, they can use male sexed semen from perhaps a higher beef producing bull such as a Limousine on the rest of their cows. This way both the highest milk and highest beef production are achievable on one operation. These systems also tend to result in lower environmental impacts than separate beef and dairy production systems, thus having an additional environmental benefit because the maintenance environmental costs of the cow herd are diluted between two products [54].

Use of Nutrition

As mentioned previously, it is important to make sure the cow is in a positive energy balance to be reproductively efficient. When there is insufficient energy to go to reproductive demands then reproduction is much more likely to fail, irrespective of the stage of reproduction during the energy shortage. Despite the widely-acknowledged ways in which under-nutrition can influence reproduction, there is also emerging literature to support the idea that targeted nutritional strategies can enhance reproduction. For example, diets high in rumen degradable protein contribute to elevated plasma urea concentrations, which decrease first service conception rate [55]. In turn, surveys find that cows with plasma urea nitrogen lower than 20 mg were three times more likely to conceive than those with higher concentrations [56]. In addition to low-rumen-degradable-protein rations, insoluble fats, calcium soaps of fatty acids in particular have also been studied for their effect on reproduction. These fatty acids contribute to elevated progesterone production and subsequently caused the cows to have a higher pregnancy rate and a reduction in days open postpartum [57]. Other relationships between short-chain fatty acids are worth additional investigation because these fatty acids are the primary product from rumen fermentation and can be fairly readily modulated by diet. For example, a higher starch diet has a higher production of short chain fatty acid propionate in the ruminant [57,58]. Our group showed that jugular infusion of propionate tended to increase progesterone concentrations in Holstein heifers [57–59]. In this same study, heifers infused with propionate had an increased insulin sensitivity index. A higher insulin sensitivity has been associated with an increase in Insulin Growth Factor 1 (IGF1) production [57–60], which aids in the resumption of cyclicity in postpartum dairy cows [61]. Conceivably these trends suggest that supplementing starch-rich feeds during the breeding period could improve reproductive success.

Individual vitamins and minerals also play an important role in reproductive success. Vitamin A deficiency has been proven to have an adverse affect on placental and fetal development in mice, making the likelihood of terminating pregnancy increase [62]. Although this relationship between Vitamin A and reproduction was apparent in mice there are mixed results with studies involving cattle with some showing positive and others showing no significant differences in regards to reproductive outcomes [63]. Vitamin E and Selenium have also been shown to improve bull and cow stress tolerance enabling improved reproductive performance during stress events such as the postpartum period or during thermal stress [64-65]. The role of Vitamin D in reproduction in cattle is not well known, however it is recommended to supplement Vitamin D in diets of cattle that have limited access to sunlight because Vitamin D plays an important role in regulating calcium homeostasis [62]. Correspondingly, Calcium plays an important role in successful calving, and low levels of calcium have been shown to cause an increase in incidences of dystocia, retained placenta, and prolapsed uterus [62]. There are a number of B vitamins that have also been linked to reproductive success. Notably Vitamins B2 and B12 have both been shown to interrupt pregnancy when insufficient in the diet [62]. Microminerals are also essential for successful reproduction. In particular, deficiency in Zinc leads to poor reproduction, as Zinc is involved in over 200 proteins and enzymes [66]. When looking at the male in particular Zinc is an important part of the membrane of the sperm cell and is beneficial to tail morphology and overall motility of the sperm cell [66]. Further investigation of vitamin and mineral effects on reproduction and other feed supplementation strategies may be beneficial in helping to find short-term management strategies that will help maximize the likelihood of reproductive success.

Conclusions

Meat and milk products are an important part of global diets; but maintaining the relevance of these products in a world increasingly aware of food insecurity and environmental impact challenges requires improving the environmental impact of beef and dairy production systems. There are a number of strategies that can be employed to enhance sustainability of ruminant production systems; however, breeding animals to feed people is among the more efficient, meaning an increase in reproductive success can have a substantial difference on cattle production efficiency as it will increase calf crop production year by year while also shortening overall calving windows and improving overall production by each cow. Through proper application of reproductive technologies, the most efficient advances in genetic gains are achieved, enabling selection of livestock that are efficient and well-adapted to their production environment. Similarly, optimizing the calves per cow per unit time ensures animal resources are used most efficiently and reduces the environmental maintenance cost of livestock production. Optimizing reproductive efficiency can be accomplished through improved genetic selection for fertility and fecundity; applying more effective use of assisted reproductive technologies; and coupling reproductive and nutritional management to optimize likelihood of reproductive success. Collectively, these approaches contribute to improved reproductive efficiency, which has been shown to enhance environmental impact of beef and dairy production systems, thus contributing to the sustainable provision of a secure global food supply.

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FIGURES

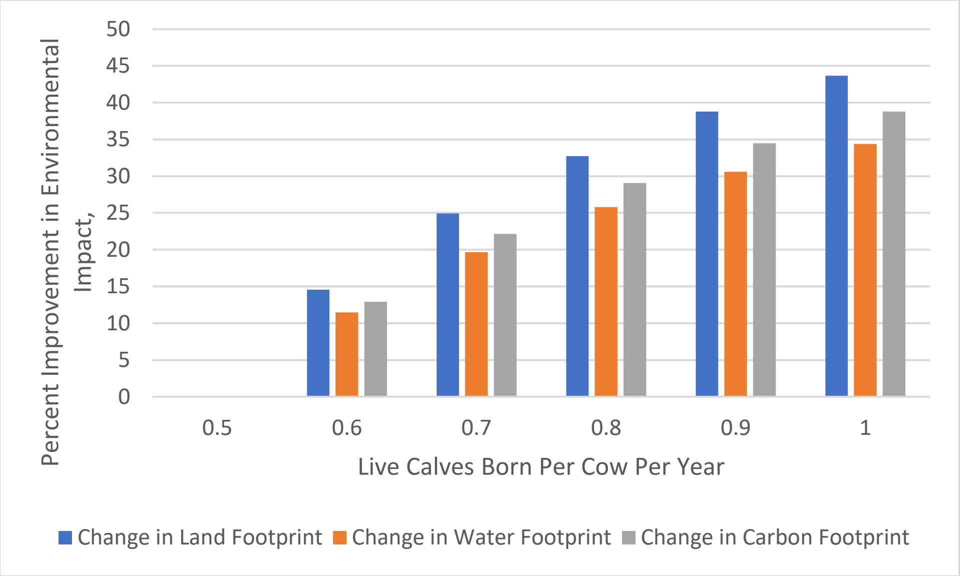

Figure 3. 1. Relative changes in environmental impact of a simulated US beef production system. Percent improvements in environmental impact use a 0.5 calf per cow per year conception rate as a baseline for calculations.

INTERPRETIVE SUMMARY. Relationships between dietary composition and reproductive outcomes in cattle, Davis. Reproduction is a common goal and costly challenge in modern dairy operations. Modulation of reproductive efficiency through dietary intervention would theoretically be a low-cost strategy to enhance dairy cattle reproduction. This paper builds on previous meta-analyses and leverages similar approaches to demonstrate that overall pregnancy rates, days to ovulation postpartum, and pregnancy rates at first artificial insemination were affected by dietary nutrient concentrations and interactions among nutrient concentrations.

RUNNING HEAD: DIET AND CATTLE REPRODUCTION

CHAPTER 4

Relationships between dietary composition and reproductive outcomes in cattle

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ABSTRACT

Individual experiments and recent literature summaries suggest links between cattle reproductive outcomes and dietary composition. However, there is limited consensus regarding how individual dietary nutrient composition or other management factors such as dairy breed, housing, and parity impact reproduction. There also is a limited consensus on the interactions among nutrients and the other management factors and their effect on reproduction. The objective of this study was to quantitatively summarize the effects of dietary composition and management factors on the reproductive efficiency of cows and heifers. A literature search returned 33 previously published papers containing 120 treatment means that were eligible for use in the meta-analysis. Diet composition information of interest included NDF, the ratio of ADF to NDF, CP, ether extract (EE), and NSC. The management factors of interest included dairy breed (Holstein and other), housing (tie stall and other), and parity. For papers to be included, they needed to be conducted using lactating dairy cows; report overall pregnancy rate, first service conception rate; or days to ovulation; fully describe experimental design details (treatments, design, number of experimental units, measurement SE or SD values, and *P*-values); and report DMI and post-calving diets with all ingredients and inclusion percentages. Details on diet composition, intake, and reproductive outcomes were extracted and analyzed using linear mixed-effects models with a random intercept for each study. When diet composition information was not reported, it was estimated from commercial feed library data and reported dietary feed inclusion rates. Days to ovulation, pregnancy at first AI, and overall pregnancy rate were the primary reproductive outcomes of interest. Significant variables were identified through a backward elimination multiple regression approach. The overall pregnancy rate model resulted in an unadjusted concordance correlation coefficient (uCCC) of 0.700 and indicated the highest predicted overall pregnancy rate at high CP, low NSC, Low NDF, and high EE. The univariate results indicated that the Holstein breed type ($P = 0.08$) and tie stall housing ($P = 0.041$) indicated a lower predicted overall pregnancy rate. The response variable pregnancy at first AI exhibited a uCCC of 0.865 and indicated the highest predicted pregnancy at first AI rate at high CP, low NSC, high NDF, and low EE. In the univariate results, the DMI ($P = 0.043$), EE ($P = 0.046$), and primiparous heifers ($P = 0.028$) all had a predicted negative relationship with the pregnancy at first AI response variable. Lastly, in the days to ovulation response variable, a greater ADF to NDF ratio is predicted to increase days to ovulation ($P < 0.001$). Additionally, greater NSC inclusion is predicted to decrease days to ovulation ($P = 0.032$).

Key Words: reproduction; nutrition; meta-analysis

INTRODUCTION

The ability for cattle to reproduce is crucial for the efficient production of low-cost meat and milk. It is well documented in the United States (US) dairy industry that reproductive efficiency continues to decline (Lucy, 2007). Ways to increase reproductive efficiency in cattle include proper reproductive management, adequate healthcare protocols, careful oversight of animal welfare, thorough genetic improvement planning, and delivery of adequate nutrition (Lucy, 2001). Despite clear recognition of its importance, there is little consensus about how or if the diet of cows pre- or post-breeding can be manipulated to enhance reproductive efficiency (Roche, 2006).

To date, both the beef and dairy industries have invested heavily in reproductive technologies and genetic improvement whilst also improving overall animal health as strategies to improve reproductive efficiency. However, diet formulation specifically to increase fertility has not been widely utilized, perhaps because of the limited consensus on the efficacy of different nutritional strategies to modulate reproduction. An additional challenge with diet formulation to increase fertility is the potential conflict with diet formulation to maximize production. If dietary strategies can be identified which improve cattle fertility, a secondary analysis is needed to determine whether such approaches would be economically beneficial, considering that they may negatively affect production. However, more recent literature has identified that reproductive success has been improving since the year 2000 (Wolfenson and Roth, 2019). These results are encouraging, however, more research can certainly be conducted to further improve reproductive success in the dairy cow. Individual animal studies to assess crude protein (CP) (Canfield et al., 1990; Triplett et al., 1995), starch (Salehi et al., 2016) and ether extract (EE) (Zachut et al., 2010; Elis et al., 2016) for their effect on reproductive outcomes have been conducted with mixed outcomes. Much of this literature is summarized in meta-analyses that investigate the effects of dietary CP (Lean et al., 2012) and EE (Rodney et al., 2015) on the fertility of dairy cattle. Such analyses explore interactions among nutrients using a meta-regression framework. Within this framework, interactions are confined to focus only on the primary driving variable of interest. A more recent paper leveraged a multi-variable meta-analysis to highlight independent, additive effects of multiple dietary characteristics on reproductive outcomes (Rodney et al. 2018). This paper found evidence of interactions among dietary components, though these interactions were not explicitly tested for in a mixed-effect linear regression. Another challenge with these existing analyses is that although they leverage contemporary dietary formulation software metrics (e.g., degradable protein and carbohydrate profiles) the results are only applicable for the single ration formulation system used. A logical next step in our understanding of the relationship between diet and reproduction is to more explicitly evaluate how concurrent shifts in multiple dietary nutrient concentrations interact to impact reproductive outcomes, and to do so using explanatory variables that can be directly derived from commonly accepted analytical procedures.

The objective of this study was to quantitatively summarize the effects of dietary composition and management factors on the reproductive efficiency of cows and heifers. We hypothesized that summarizing the literature available would reveal key relationships among dietary nutrient concentrations, feed intake, management factors, and reproductive outcomes that were not previously identified in meta-analyses that focused on unilateral shifts in specific nutrients.

MATERIALS AND METHODS

Data Collection

The publication search began in September of 2018 and ended in March of 2019. Data were obtained from 33 previously published articles containing 120 treatment means that were eligible for use in the meta-analysis. These papers were identified using a systematic review process following the guidelines of Khan et al. (2003). The first step was to identify the research questions that should be answered within the review. This included assessing what variables of interest we wanted to study and to identify our criteria for literature inclusion into the database. The second step of the systematic review process was to identify relevant work. For papers to be included within the dataset, they needed to be conducted using lactating dairy cows; report overall pregnancy rate, first service conception rate; or days to ovulation; fully describe experimental design details (treatments, design, number of experimental units, measurement SE or SD values, and *P*-values); and report DMI and post-calving diets with all ingredients and inclusion percentages. We utilized only dairy studies because few studies on beef cattle met the inclusion criteria, creating an imbalance within the dataset. We also excluded articles that had non-typical reproductive technologies such as embryo transfer or *in vitro* fertilization. These articles were sourced from different journals, but approximately 70% were from the Journal of Dairy Science. Journal articles were identified by searching Google Scholar, Pubmed, and CAB using combinations of the keywords: protein, starch, NDF, ADF, fat, cattle, cow, heifer, reproduction, fertility, pregnancy, management, dairy, and conception. As articles were identified for inclusion, their references were screened to identify additional related studies not identified by the literature search. The third step of the systematic review process was to assess the quality of the studies. This was achieved through rigorous screening of the papers by evaluating their study design, response variables, animals used, and several other factors to ensure sure they fit our criteria and that there were no repetitions among selected papers.

The fourth step was to extract the data from the included studies. From the 33 published articles that were identified for inclusion, data on the following variables were transcribed into a spreadsheet: complete diet ingredients, ingredient inclusion percentages, DMI, breed, parity, housing information (tie stall, free stall, grazing, among others), authors, statistical method, experimental design, number of cows in each experimental unit, and body weight (if reported). When recording data, breed was separated into 2 categories: Holstein or other dairy breeds. Parity was separated into two categories: multiparous or primiparous. Studies that reported combined data for multiparous and primiparous cattle were coded as multiparous. Housing type was separated into two categories: tie stall (if cows were kept tied in a stall) and free stall (if cows were in open lots or free stalls). Studies reported on a wide variety of reproductive outcomes; however, the three selected for analysis due to highest reporting rates were: days to ovulation, overall pregnancy rate, and pregnancy at first artificial insemination (AI). Higher pregnancy at first AI and overall pregnancy rate were desirable, whereas lower days to ovulation was more desirable because the animal would be coming back into heat more quickly.

Data Cleaning

Data entry errors were assessed by independent review of the literature and the developed database. This review was conducted by two individuals independent of the person responsible for original data entry. Additionally, outliers were screened using univariate and multivariate graphical assessments. When density and correlation plots revealed data obviously different from the overall grouping or trend, the original papers were checked to ensure values were recorded accurately. Data were only amended if entry errors were made, no outliers were identified for removal prior to analysis.

Diet Composition Calculation

Diets reported from qualified publications were obtained and entered into the database. Once diets were recorded with their respective inclusion percentages of each feedstuff, missing feedstuff nutrient composition information was filled in using feed composition data from The National Animal Nutrition Program, Feedipedia, and Dairy One feed composition databases, in that order. Ingredient composition data that were filled in, if not reported in the paper, included: neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), ether extract (EE), and nonstructural carbohydrates (NSC). Because ADF and NDF are closely related, a ratio of

ADF to NDF was utilized in the model in place of ADF as a main effect. If EE was not reported then the crude fat percentage was considered in place of EE. All articles included reported either EE or crude fat.

Model Derivation Procedure

The last step of the systematic review process was to interpret the findings. To do this we used R version 3.5.1 (R Core Team., 2014) for model derivation. Study was included in every model as a random intercept effect. Univariate models were used to test the linear associations among explanatory and response variables. Although these assessments have limited practical interpretability, they can help demonstrate the directionality of gross relationships and can be used as a tool for understanding confounding and collinearity. Significant relationships were identified at $P \leq 0.05$ and tendencies were declared at $P \leq 0.10$.

Multivariate models were also derived. In the initial model, multivariate models considered all explanatory variables, including DMI, and selected 2-way interactions. The 2-way interactions were selected by having an interaction of each explanatory variable, in the initial model. To find significant interactions and the main effects, the backward elimination multiple regression procedure was used following the method described in Gleason and White (2018). In brief, the initial model included all variables and their interactions that could affect the priority response variables mentioned previously. Both dietary explanatory and reproductive response variables are identified in Table 4.1. The other explanatory variables including dairy breed, housing, and parity are identified in Table 4.2. Explanatory variables and their interactions were removed in a step by step procedure by iteratively eliminating the highest P-value variable one by one until there was only significant ($P \leq 0.05$) or trending ($0.05 < P \leq 0.10$) P-values present.

Linear effects above P>0.10 were retained in the model if the corresponding interaction terms were P≤0.10. The response variable Days to Ovulation failed to converge due to the small number of complete observations of the inclusion of interactions, therefore only a univariate model was utilized. The explanatory variables: breed, parity, and housing type also a part of the initial model with their interactions between each other and dietary compositions.

Both the univariate and multivariate models were weighted by the number of animals included for each explanatory variable, which is not a standard practice. Most meta-analyses are weighted by 1/standard error (SE) reported with the study because this weighting strategy best reflects the known variance associated with each study. However, SE of reproductive outcomes is rarely reported because these outcomes are often measurements on a population of animals (e.g., % pregnant) and not reflective of values summarized across a population of animals. Because these studies often report summary statistics for a population there is no replication, and therefore no reliable measures of variation. Although this approach is changing in more recent reproduction literature, the meta-analysis includes a large amount of older literature where SE was not reported. All studies used in the analysis used some variation of a completely randomized design, and the number of animals in each treatment should be the best available representation of the confidence in the reproductive variables measured on those animals. Therefore, we chose to weight by the number of animals, even if it not the ideal protocol.

To measure the severity of multicollinearity and to assess the extent that parameter error variance is magnified due to collinearity, variance inflation factors (VIF) were calculated. The square root of the VIF demonstrates the magnification of the variance of the parameter estimates when compared with what would be optimal (no collinearity). Established cutoffs for VIF are not published, however we used values outlined by (Roman-Garcia et al., 2016), and variables with VIF<10 were removed from models, unless they were part of an interaction term.

Evaluation of Model Performance

Although the root mean square error (RMSE) is often used as a model evaluation tool in meta-analyses, it is not appropriate because the models are usually not derived using least-squares approaches. As such, model fit was evaluated using the root estimated variance associated with study and error. We also assessed the concordance of the model fit as measured by Lin's concordance correlation coefficient (Lin, 1989). The study-specific calibration for the coefficients (random intercepts) was assumed unavailable so these were not used in calculating CCC (termed uCCC). Importantly, the intent of evaluating model fit was not for comparison among models but rather, to understand the degree of variation in the response that was explained by the linear association(s) with the explanatory variable(s).

RESULTS AND DISCUSSION

The distribution of breed, parity, and housing variables, and the summaries of dietary composition and response variables are included in Table 4.1 and Table 4.2. All 120 treatment means included dietary composition; however, not every treatment mean included all three response variables of interest. A total of 57 treatment means reported overall pregnancy rate, 45 reported pregnancy at first AI rate, and 34 reported days to ovulation for dairy breed types.

Overall Pregnancy Rate

Univariate Model. In the univariate models, overall pregnancy rate was significantly affected by the research housing system used (*P* = 0.041). The reference level of housing type was a free stall barn. As such, the coefficient applied in the model reflects the expected change in reproductive parameters when using tie stalls rather than free stalls. Previous research suggests tie stalls may inhibit animals from expressing natural behaviors (Rousing et al., 2004). Bielfeldt et al. (2005) also found that cows in tie stalls without exercise exhibited a higher incidence of lameness than those in free stalls. In addition to these changes in animal behavior and locomotion, Odore et al. (2011) found that cortisol levels were significantly higher in beef bulls housed in tie stalls versus free stalls. Although dairy cattle may be more accustomed to aspects of the tie stall housing than beef animals, the fact that our meta-data suggest tie stall housing has a negative effect on overall pregnancy rate (Table 4.3) provides further evidence of potential negative physiological, wellbeing, and management implications of this housing system. Particularly when dealing with broad categorical variables such as housing system, there is always the potential for inadvertent covariation with other explanatory factors. However, in the case of the relationship between housing system and reproduction, there are potential physiological linkages that support the relationship. Elevated cortisol levels have been shown to suppress the LH pulse frequency (Breen et al., 2005), which can lead to suppression of normal breeding behaviors and may impede heat detection. Furthermore, decreased LH secretion and pulsatility induces a negative feedback loop, impairing follicle growth due to suppression of estradiol production (Dobson and Smith, 2000). Although data on cortisol responses of dairy cattle in tie stalls are limited, if their physiological responses are similar to the beef study highlighted above, this physiological cascade supports the negative impact of tie stalls on reproductive responses. The association between housing and reproduction was only identified for overall pregnancy rate, and additional research is likely required to confirm this association and evaluate potential causative mechanisms.

Overall pregnancy rate also tended to be affected by breed type (*P*=0.08; Table 4.3). Holstein animals are the reference for the breed variable, meaning that the coefficient in the model reflects the use of Holsteins rather than Jerseys or other breeds. This univariate assessment suggested Holsteins have lower overall pregnancy rates than non-Holstein breeds (Table 4.3). The Holstein breed is widely regarded as having the highest potential of dairy production efficiency among cattle (Tuzov et al., 2018); however, that high degree of milk production is recognized as negatively genetically correlated with reproductive performance (Hoekstra et al., 1994). In support of the relationship identified in our univariate models, Dillon et al. (2003) found that non-Holstein breeds had greater reproductive performance than Holstein breeds, including higher overall pregnancy rates. As such, the association between overall pregnancy rate and breed type appears consistent with the existing literature studying these relationships.

Multivariate. The significant and trending factors for the multivariate model of overall pregnancy rate are reported in Table 4.4. These relationships resulted in a relatively strong uCCC of 0.700 (Table 4.4), likely partially because of the number of significant variables versus the size of the dataset. Of those variables included in the initial model, the final model retained information on diet, housing system, breed, and parity, highlighting reproduction as a complex phenotype subject to genetic by management and time interactions. In addition to the factors highlighted as significant within these models, additional critical factors expected to influence overall pregnancy rate include: cow age, the AI technician skill, semen straw viability, among others. Because of inconsistent reporting within the literature we were unable to account for such management differences within the present analysis.

Perhaps most interesting within the relationships highlighted by the model are the numerous interactions among diet, parity, genetics, and housing system. Crude protein in particular, was involved in several interactions (Table 4.4). Previous work has highlighted discrepancies in the relationship between CP and reproductive outcomes. For example, Barton et al., (1996) showed no significant differences in pregnancy rate in Holstein cows, and heifers fed either a 13% or 20% CP diet. In contrast, McCormick et al., (1999) found a significant decrease in pregnancy percentages with Holstein cows having a 53.4% and 75.4% pregnancy rate fed 23.1% and 17.7% CP respectively. Canfield et al., (1990) also found a significant decrease in first service conception rates of Holstein cows and heifers fed either a 16.5% and 19.2% CP diet with 7.31% and 8.04% rumen undegradable protein, respectively. Canfield et al., (1990) found both the cows and heifers had a 35% decrease in the first service conception rate when CP was increased, though, notably the first service conception rate is not the same as overall pregnancy rate. These discrepancies among experiments evaluating the relationship between dietary protein supplies and reproduction could be partially explained by interactions with other dietary and non-dietary factors, such as those highlighted within the present model.

Figure 4.1 shows the predicted overall pregnancy rates of multiparous cows when feeding a variety of dietary scenarios reflecting changes in NDF, CP, EE, and NSC. On low NDF diets, transitioning from 14 to 16% CP would be expected to dramatically decrease overall pregnancy rates; however, at high NDF levels, the impact of increasing protein is less severe. The relationship between overall pregnancy rate, NDF and CP is more consistent at moderate levels of fat. As EE increases, adding additional protein becomes more advantageous whereas at low levels of EE, adding additional protein becomes contradicted for reproductive success; however, this response can be partially rescued by elevated NSC. The severity of these interactions becomes more pronounced when evaluating primiparous animals (Figure 4.2). Although these interactions suggest diet has complex effects on reproduction, we can simplify the associations to reflect interactions between energy, energy type (volatile fatty acids vs glucose vs fat), and protein supply. When energy supplies are lower (reflected by elevated NDF), reproductive success was compromised in all cases. Due to the energetic demands of the lactating dairy cow (Harvatine and Allen, 2006) and the notorious challenges associated with bringing a cow back into reproductive cyclicity while sustaining high levels of milk production (Lamming and Darwash, 1998; Opsomer et al., 1998), it is not surprising that this association with elevated NDF and reduced overall pregnancy rates was identified across other factors.

A particularly biologically interesting interaction with parity is linked to the idea of energy supplies. Notably, primiparous animals benefited from addition of fat or NSC into the diet; however, multiparous animals were negatively impacted by elevated NSC. The high pregnancy rates related to moderate CP with low NSC for multiparous cows and moderate CP with high NSC for primiparous heifers observed in Figure 4.1 and Figure 4.2 corroborates previously identified relationships between carbohydrate supply fermentability and reproduction. Consisting mainly of starch and soluble sugars, NSC is rapidly fermentable (Beauchemin et al., 1997) and provides an important energy source for high producing dairy cattle to meet their energy demands and service other physiological functions such as reproduction (Reynolds, 2006). A diet high in NSC has been related to an elevated IGF-1 blood plasma concentration (Houseknecht et al., 1988). Estradiol concentrations have been highly correlated to plasma IGF-1 levels (Spicer and Chamberlain, 1998), meaning elevated IGF-1 is beneficial to reproductive outcomes. Because of the importance of starch in providing energy for dairy cattle, it is conceivable that an increase in NSC would be beneficial to reproduction, particularly for energystarved physiological stages such as primiparous cows which are also still growing. This idea is supported by experimental literature, for example, Burke et al., (2010) found increasing NSC improved first service conception rate. However, high NSC diets can also be associated with a higher risk of acidosis and other metabolic diseases (Reynolds, 2006). As such, multiparous cows, which are not subject to the additional energy requirements for growth, may, on average, benefit from more conservative NSC feeding strategies focused on low risk of metabolic disease.

Both Figure 4.1 and Figure 4.2 suggest additional interactions among CP and NSC. Namely, at low NSC, adding CP reduced reproductive success whereas the opposite occurred at high NSC. This phenomenon could reflect the need for matched fermentability in carbohydrate and protein profiles to optimize the rumen environment, and by association subsequent physiological systems. However, there is additional experimental work suggesting other physiological mechanisms for the interaction of protein and NSC. For example, Rooke et al. (2009) found that a high starch supplementation was detrimental to oocyte quality, unless leucine was supplemented. Indeed, the differences observed between the high and low starch groups in Figures 4.1 and 4.2 support the idea that elevated protein supply is required to prevent negative impacts of high NSC on overall pregnancy rate. Additional experimental work is needed to better characterize the extent and importance of interactions among carbohydrate and protein supplies as they affect reproductive outcomes.

The effect of EE on reproduction was positive across all scenarios but the magnitude of impact differed substantially. This interaction with other dietary components could partially explain some of the discrepancies with the scientific literature evaluating the associations among dietary fat and reproductive outcomes. For example, although Fuentes et al. (2008) found no significant difference in pregnancy rates when supplementing linseed to a total mixed ration, Sklan et al., (1994) found that increasing the concentration of EE in the diet decreased first service conception rate by 40.4%. By contrast, de Veth et al., (2009) found an increase in pregnancy rates when EE was supplemented in their multi-study analysis. In a study evaluating very extreme levels of fat, Scholljegerdes et al., (2009) found that cows fed a 26% EE diet exhibited a lower pregnancy rate to AI when compared to cows fed a 2% EE diet. Because increasing fat (indeed, any nutrient) comes at the expense of trade-offs in other dietary nutrients, the collateral nutrient shifts among these studies, along with the significant interactions among EE and other dietary parameters highlighted within our model, may partially explain the differences in experimental results observed previously.

Another potential mechanism governing the highlighted relationships between reproductive outcomes and EE are physiological impacts of EE on embryo quality and development. For example, one study fed lactating dairy cows either 200 or 800 g ⁄ day of Calcium long-chain fatty acids of palm oil. Follicles were then aspirated, matured, and fertilized from each cow. A greater percentage of oocytes developed into blastocysts from cows fed the high-fat diet and those blastocysts had more total cells which are indicative of higher quality (Fouladi-Nashta et al., 2007). In most scenarios evaluated within the present work, addition of EE improved reproductive outcomes. Although the benefit of added EE could be attributed to improved energy balance, these studies demonstrating links between follicle quality and maturation and dietary EE composition could also support the positive association.

Pregnancy at first AI

Univariate. In the univariate model pregnancy at first AI was significantly affected by DMI (*P* = 0.043), EE (*P* = 0.046), and parity (*P* = 0.028; Table 4.3). Although, depressed DMI is often associated with impaired health (Huzzey et al., 2007), our work identified that elevated DMI resulted in lower pregnancy at first AI. This decrease in pregnancy at first AI is likely due to the covariation between DMI and performance level. Elevated DMI supports high milk production through improved energy balance (Grummer et al., 2004); however, there is a negative genetic correlation between milk production and reproduction. As such, this effect is likely reflecting the fact that high producing cows have elevated DMI, but often also have impaired reproductive success.

Our model also predicted a negative association between EE and pregnancy at first AI. This response is contradictory to most of the scenarios used in evaluating the overall pregnancy rate model, which largely found improved overall pregnancy rates with elevated EE. That said, the decreased reproductive performance with elevated EE is consistent with some of the literature; through results have been variable (Staples et al., 1998). A potential physiological explanation for the difference detected between reproductive responses at first AI and overall may be due to the physiology of animals early in the rebreeding window. Early in this window, animal likely still have depressed DMI. Theoretically, this depressed DMI with a higher amount of EE inclusion in the diet could lead to substantial biohydrogenation of fats, which may negatively impact digestibility and dietary energy utilization (Rabiee et al., 2012). However, the discrepancies within the literature and within this meta-analyses support the need for further research to evaluate how different fatty acid profiles and basal diets interact to influence reproduction throughout the production cycle.

The final significant univariate relationship identified was with parity, suggesting primiparous cows had lower pregnancy rates at first AI. Numerous studies have characterized improved pregnancy rates in heifers when compared to lactating cows (Lee et al., 1983; Pursley et al., 1997); theoretically because of the lactation energy demands and age, among other factors. However, Pursley et al. (1997) found a lower pregnancy rate in heifers when compared to cows when the animals were subject to an Ov Sync protocol, possibly due to differences in the uterine environment and oocyte quality among age groups. The lower first AI pregnancy rate of primiparous cows versus multiparous cows identified in the present study could also be simply reflective of poorly balanced data relative to heifer feeding (Table 4.3).

Multivariate. Much like overall pregnancy rate, pregnancy at first AI was significantly affected by interactions among diet, housing system, parity, and genetics (Table 4.4). These relationships resulted in a relatively strong uCCC of 0.865 (Table 4.4), though the strength of this model could also reflect overfitting given that the dataset is fairly limited. Much like the overall pregnancy group, there were several interactions which complicated the interpretation of results, meaning scenarios can be a more effective way to evaluate patterns represented within the model.

The expected first AI pregnancy rate responses to dietary nutrient changes differed from those identified in the overall pregnancy model, as observed in Figure 4.3. There was not an included figure that investigated the relationship between primiparous heifers and pregnancy at first AI due to limited treatment means and illogical estimated response values. The results in Figure 4.3 suggest the conditions that lead to successful rebreeding early in the rebreeding window may differ from those conditions which contribute to successful breeding overall or later within that window. Notably, the directionality of the response to NDF differed between the response variables, with first AI pregnancy rate increasing with increasing NDF content. This benefit of higher NDF content earlier in the rebreeding window may be associated with the elevated incidence of metabolic diseases which can occur in the transition period (Curtis et al.,

1985) because higher fiber diets are associated with reduced incidence of metabolic disorders such as acidosis by helping to regulate ruminal pH (Bernard and McNeill, 1991).

Another notable difference between the overall pregnancy rate model and the first AI pregnancy rate model is the relationship with EE. The first AI pregnancy model suggested high EE was beneficial at lower CP content but detrimental at higher CP content, and the point at which EE addition changes from benefit to detriment was dependent on NSC concentration in the diet. Increasing EE in diets, within a moderate range, improves energy balance (von Soosten et al., 2012), pregnancy rates (de Veth et al., 2009), and a decreased incidence of metabolic disorders (Rodney et al., 2015). Additionally, diets higher in n-3 fatty acids can improve endometrial PGF2a production (Santos et al., 2008). Although diets higher in n-6 fatty acids have been shown to improve progesterone concentrations (Santos et al., 2008). Sklan et al., (1991) showed addition of calcium soaps of fatty acids to the ration significantly increased the percentage of cows pregnant at 150 days. Staples et al., (1998) also suggested that diets with at least 3% DMI fat supplementation in a ration increase ovulatory follicle size, number of follicles, the plasma concentration of progesterone, the overall lifespan of corpus luteum, and improve fertility. As such, the higher first AI pregnancy rates associated with elevated EE on lower CP and higher NSC diets is consistent with the previous literature. The change in the response to fat at lower NSC and higher CP levels is somewhat paradoxical; however, the slope on NDF in these diets is also steeper than on lower CP diets. These behaviors may cumulatively suggest that higher CP diets contribute to the metabolic sensitivity of animals during the early portion of the rebreeding window. If indeed that is the case, it may also help explain some of the discrepancies in the existing literature on CP feeding and reproductive outcomes.

In general, few studies reported both first AI pregnancy rate and overall pregnancy rate. The differences in responsiveness to dietary composition between these two reproductive outcomes suggests additional research should focus on feeding strategies tailored to rebreeding success dependent on physiological stage. Specifically, it would be beneficial to try and design studies to isolate the potential inhibitory effects of metabolic disease complications from any potential beneficial effects of nutritional strategy early in the rebreeding window. Such studies could help identify whether responses to diet are due to preventing or decreasing incidence of metabolic diseases, or whether there are independent diet-specific benefits such as provision of amino acids, essential fatty acids, total energy supply, etc.

Days to ovulation

Univariate model. The experimental methodology used to measure days to ovulation varied across included studies. For example, Canfield et al., (1990) and Carroll et al., (1989) predominately used visual estrus assessment, while Bruckental et al., (2000) determined days to ovulation by assessing when plasma progesterone concentrations reached >1 ng/ml. In the days to ovulation univariate models, significant relationships with NSC ($P = 0.032$) and the ADF:NDF ratio ($P < 0.001$) were identified. Days to ovulation decreased with elevated dietary NSC (Table 4.3). When compared to structural carbohydrates, NSC is a more readily available form of energy (Visser et al. 1992) because the starches and sugars present in NSC are easily and quickly digested and absorbed from the rumen (Visser et al. 1992). The ease of digestibility aids in the resumption of cyclicity by improving plasma glucose concentrations, and subsequently improving plasma IGF-1 concentrations more efficiently (Nicolini et al. 2013). This increase in plasma IGF-1 concentration aids in the cow’s ability to resume cyclicity and come back into heat, thus lowering the days to ovulation. Given the energetic benefits and downstream effects

NSC can have on reproductive hormones, the results seen in Table 4.3 are not unexpected. It is worth noting; however, that they are somewhat contradictory to the importance of controlling metabolic disease suggested by the pregnancy at first AI models. As discussed previously, there are substantial opportunities for future work to clarify some of these relationships.

Our model also predicted that increased ADF:NDF ratio was associated with increased days to ovulation (Table 4.3). This is sensible given that a greater ADF:NDF is reflective of lower hemicellulose relative to lignin and cellulose (Beauchemin, 1996), which is associated with reduced NDF digestibility (Robinson and Erasmus, 2009). The negative energy balance post-calving (Wathes et al., 2007) increases days to ovulation, and further exacerbating this negative energy balance through impaired NDF digestibility likely contributes to delayed reproduction.

Limitations and Future Opportunities

The models derived in this study provide a view on the complexity of interactions between diet, management, and reproduction. The relationship between diet and reproduction is clearly complicated by physiological stage, genetics, and management. Although the models derived in this work are unlikely to be useful in formulating rations for enhanced reproductive outcomes, or for economic assessment of this type of ration formulation, they do provide numerous targets for future work. The apparent discrepancies between overall pregnancy rate and first AI pregnancy rate warrant further investigation to better isolate potential explanatory or confounding factors like metabolic disease, physiological status, diet, and parity. Furthermore, each interaction identified within the work warrants more thorough experimental testing given that few, if any, studies have been designed to specifically target multiple concurrent interactions among dietary nutrition composition and their effect on reproductive outcomes. The expansion of experimental data on these relationships will provide useful corroboratory data for use in model refinement and reevaluation.

TABLES

Table 4. 1. Descriptive statistics to summarize the dietary components of transition diets and reproductive outcomes in dairy cattle.

Variable	N[a]	Mean	SD	Minimum	Maximum
Dietary composition[b], % DM					
CP	120	19.61	3.87	12.18	36.39
NSC	120	31.03	7.09	12.82	53.2
NDF	120	32.35	6.56	12.62	52.74
EE	120	5.05	2.13	2.63	19.86
ADF:NDF	120	0.63	0.05	0.49	0.77
Reproductive response variables					
Overall PR[c], %	57	61.31	18.77	0	100
First AI PR[c], %	45	42.18	16.12	14.4	82
Days to ov[d], d	34	37.63	19.68	16	85.2

a N = number of treatments

b CP = crude protein; NSC = nonstructural carbohydrate; ADF = acid detergent fiber; NDF = neutral detergent fiber; EE = ether extract

c PR = pregnancy rate

d ov = ovulation

Table 4. 2. Descriptive statistics to summarize the explanatory variables other than diet utilized in the initial models.

Factor	N[a]	N[b]
Breed type		
Holstein	102	30
Non-Holstein	18	3
Housing type		
Tie stall	24	7
Free stall	96	26
Parity		
Multiparous	105	26
Primiparous	15	7

a N = number of treatments

b N = number of studies

Table 4. 3. Univariate results of dietary components, breed type, parity, and housing conditions on reproductive response variables in cattle.

Overall pregnancy rate. %				Pregnancy at first AI, %				Days to ovulation, d			
Factor[1]	Slope	uCCC[2]	*P*-Value	Factor[1]	Slope	uCCC[2]	*P*-Value	Factor[1]	Slope	uCCC[2]	*P*-Value
Intercept	79.16	0.184	0.08	Intercept	76.18	0.286	0.043[a]	Intercept	-57.6	0.531	<0.001[a]
Holstein	-17.86			DMI	-1.52			ADF:NDF	143.12		
Intercept	68.32	0.236	0.041[a]	Intercept	65.14	0.194	0.046[a]	Intercept	74.46	0.395	0.032[a]
Housing	-19.28			EE	-4.34			NSC	-1.13		
				Intercept	61.8	0.229	0.028[a]				
				Parity	-18.87						

a *P*≤0.05.

b 0.05>*P*≤0.10.

1 DMI= dry matter intake; EE= ether extract; NSC= nonstructural carbohydrate; NDF= neutral detergent fiber; ADF: acid detergent fiber; ADF:NDF=ADF to NDF ratio; Housing= tie stall system; Parity= primiparous heifers; Breed= Holstein breed type.

2 uCCC= Lin's concordance correlation coefficient.

Table 4. 4. Multivariate results of dietary components, breed type, parity, housing condition, and their interactions on reproductive response variables in cattle.

Overall pregnancy rate, %			Pregnancy at first AI, %		
Factor[1]	Slope	*P*-Value[a]	Factor[1]	Slope	*P*-Value[a]
Intercept	2158.3	0.034[a]	Intercept	1805.15	0.253
DMI	27.81	0.022[a]	Breed	131.75	0.017[a]
NDF	-24.75	0.08[b]	DMI	165.53	0.005[a]
Breed	-329.59	0.076[b]	CP	-320.54	0.010[a]
CP	-171.32	0.004[a]	EE	282.53	0.045[a]
EE	-65.64	0.009[a]	NSC	-34.64	0.116
NSC	-19.82	0.148	Parity	1054.86	0.252
Parity	560.43	0.25	NDF	-3.95	0.814
Housing	-154.12	0.008[a]	Housing	-2084.54	<0.001[a]
DMI X NDF	-0.93	0.017[a]	ADF:NDF	-5091.01	0.025[a]
Breed X CP	16.66	0.091[b]	DMI X CP	-3.75	0.007[a]
EE X Parity	-13.61	0.010[a]	DMI X EE	-5.43	0.006[a]
NSC X Parity	-14.92	0.035[a]	DMI X NSC	-2.29	0.007[a]
EE X Housing	22.82	0.021[a]	CP X Parity	69.01	0.005[a]
NDF X CP	2.34	0.008[a]	Parity X NDF	-67.56	0.006[a]
CP X NSC	1.91	0.009[a]	NDF X Housing	32.66	0.001[a]
CP X EE	4.12	0.003[a]	Housing X ADF:NDF	1516.11	0.005[a]
			EE X Housing	20.61	0.076[b]
			CP X NDF	3.62	0.007[a]
			CP X ADF:NDF	259.29	0.023[a]
			CP X NSC	1.48	0.061[b]
			CP X EE	3.33	0.048[a]
			EE X NSC	2.16	0.022[a]
			NSC X ADF:NDF	92.25	0.006[a]
			EE X ADF:NDF	-493.55	0.011[a]
uCCC[2]	0.7		uCCC[2]	0.865	

a $P \leq 0.05$.

b $0.05 > P \leq 0.10$.

1 CP= crude protein; ADF= acid detergent fiber; EE= ether extract; NSC= nonstructural carbohydrate; NDF= neutral detergent fiber; ADF:NDF=ADF to NDF ratio; Housing= tie stall system; Parity= primiparous heifers; Breed= Holstein breed type.

2 uCCC= Lin's concordance correlation coefficient.

FIGURES

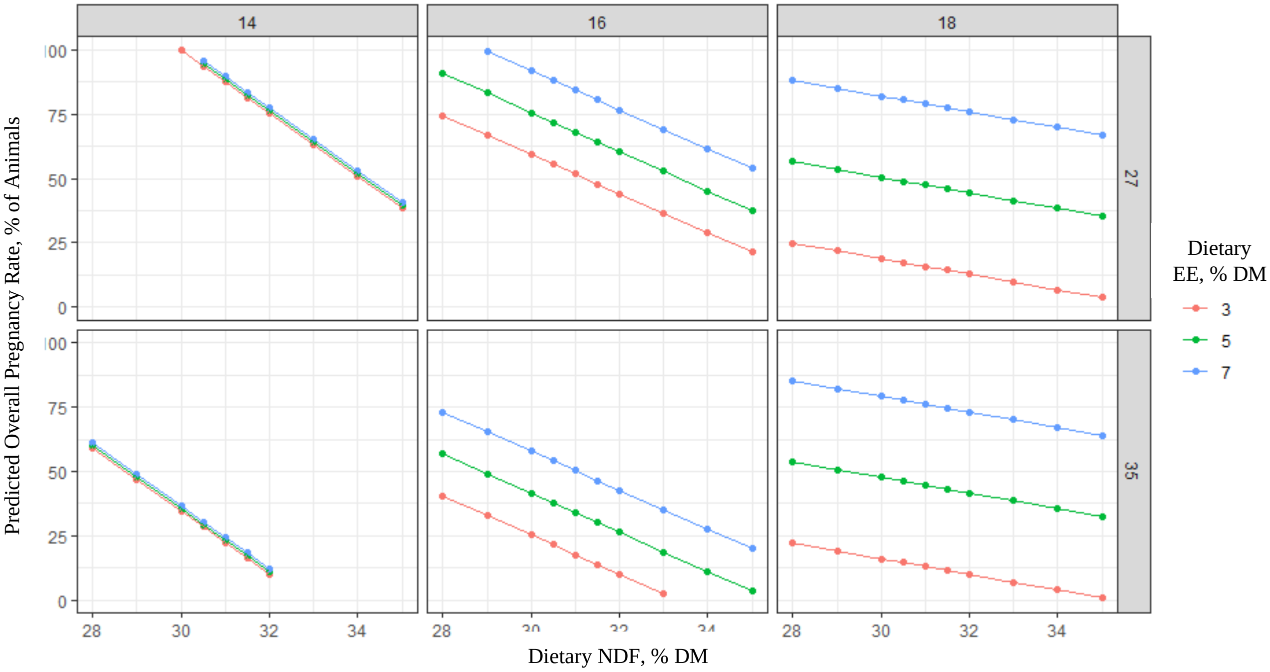

Figure 4. 1. Interactional effects of dietary inclusion percentages of neutral detergent fiber (NDF) (% diet DM, bottom X axis), crude protein (% diet DM, columns), nonstructural carbohydrates (% diet DM, rows), and ether extract (% diet DM, line colors) of multiparous cows.

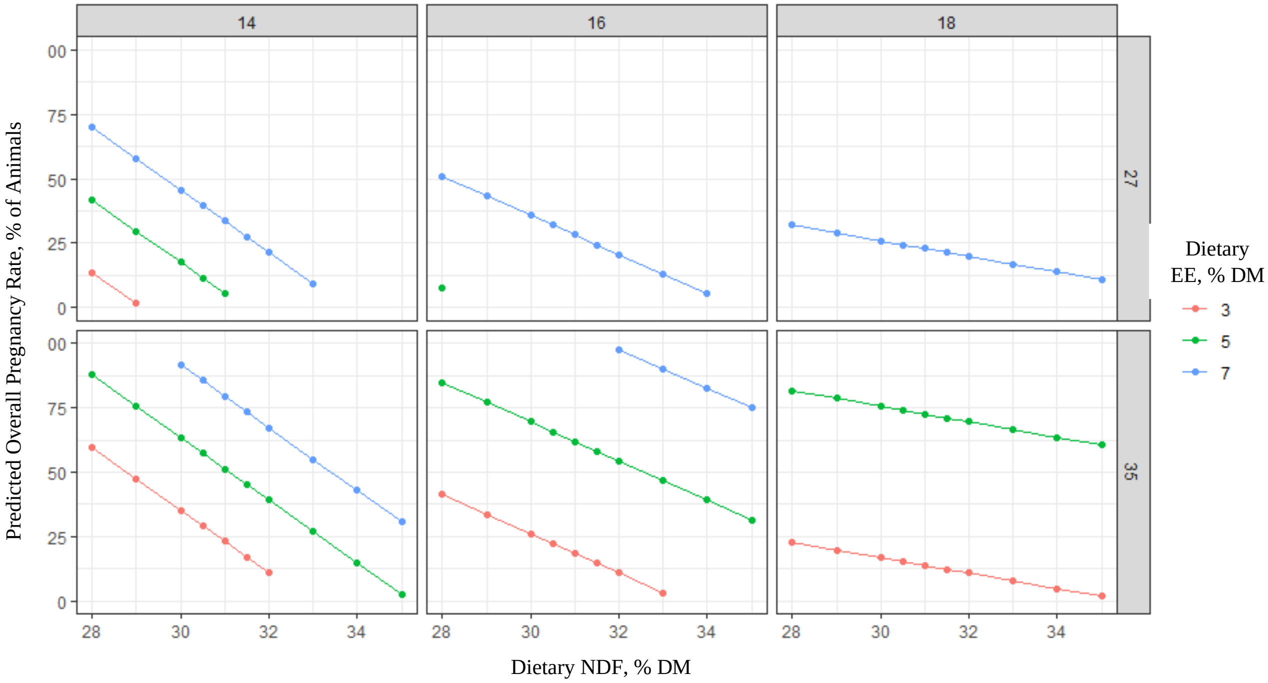

Figure 4. 2. Interactional effects of dietary inclusion percentages of neutral detergent fiber (NDF) (% diet DM, bottom X axis), crude protein (% diet DM, columns), nonstructural carbohydrates (% diet DM, rows), and ether extract (% diet DM, line colors) of primiparous heifers.

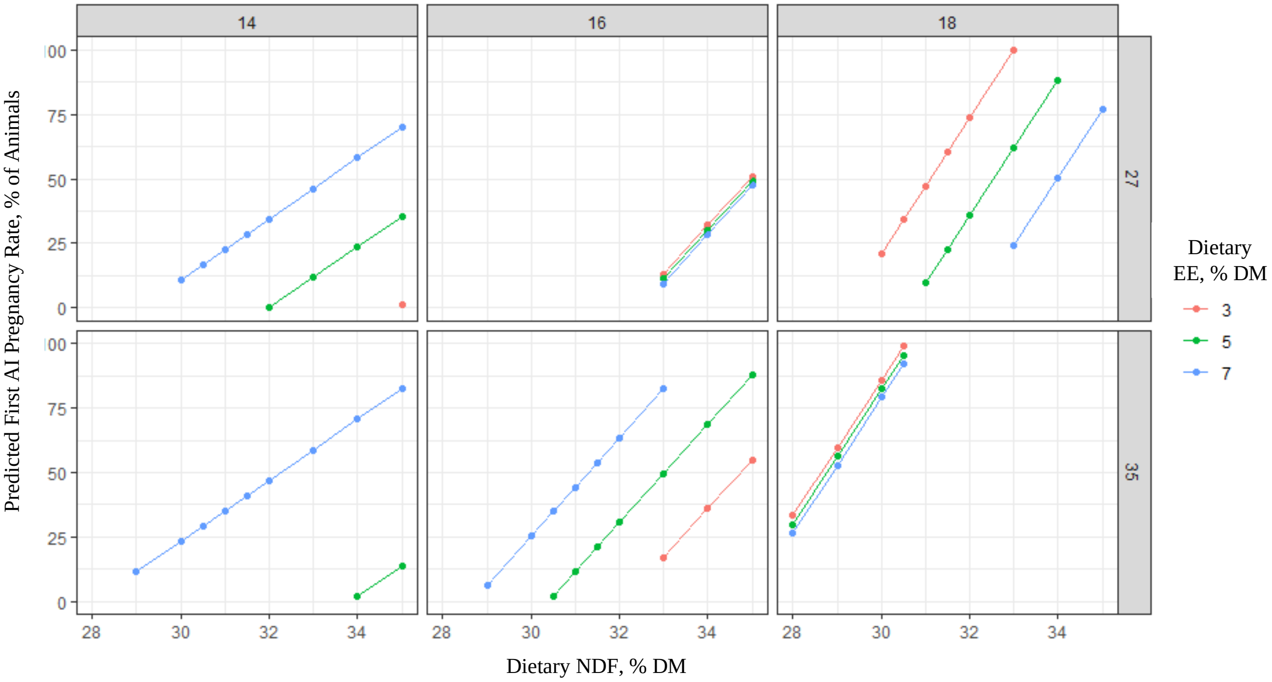

Figure 4. 3. Interactional effects of dietary inclusion percentages of neutral detergent fiber (NDF) (% diet DM, bottom X axis), crude protein (% diet DM, columns), nonstructural carbohydrates (% diet DM, rows), and ether extract (% diet DM, line colors) on the predicted first AI pregnancy rate (left Y axis) of multiparous cows.

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CHAPTER 5

This chapter is to be submitted to the Journal of Animal Science as: Effect of dietary energy source on pregnancy rates and reproductive physiology of pastured beef heifers

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ABSTRACT

Increasing dietary glucogenic precursors may improve progesterone (P4) production and conception rates in ruminants. The objective of this study was to evaluate how isoenergetic diets leveraging starch or fat as the primary energy source affected pregnancy rates, follicle size and number, placental-associated glycoprotein (PAG) concentrations, and growth rate of pastured heifers. Breeding-age Angus cross heifers (n = 29; Age: 12 mo; BW = 310 ± 79.8) were allowed *ad libitum* access to hay and water. Animals were balanced by age and BW and randomly assigned to treatment grain mixes, which were fed through an automated feeder at a maximum rate of 4.54 kg/d. Experimental grain mixes had similar calculated ME (0.97 kg/d) and MP (1.13 kg/d) contents and included a high starch (47.5% starch) diet and a high fat (11.5% fat) diet. Experimental diet feeding began 8 d before initiating a 7 d Cosynch + CIDR protocol synchronization protocol, TAI, and pregnancy diagnosis at 52 days post-first AI. Follicle measurements, including the number and size of dominant follicle(s), were collected by rectal palpation ultrasonography on days -10, -8, -4, and day 0 relative to breeding. Plasma samples for progesterone analysis were collected on days -10, 1, 2, 3, 4, 5, 6, 7, 10, 14, 18, 21, 24, and 28, relative to breeding, and PAG concentrations were determined on days 24 and 28. If animals showed heat after the first insemination on d 0, they were rebred 12 h after standing estrus and samples reflecting days 10, 14, 18, 21, 24, and 28 post-breeding were re-collected and assayed for progesterone and PAG. All animals were checked for pregnancy after or on day 28 after their first or second AI by ultrasonography. No treatment differences in final BW (*P*= 0.58), final BCS (*P*= 0.419), ADG (*P*= 0.106), hay DMI (*P*= 0.64), and total DMI (*P*= 0.418) were observed. The starch grain mix showed a numerically greater first (53.3% vs. 42.9%) and second (83.3% vs. 61.5%) AI conception rate. The starch diet resulted in a tendency for greater PAG concentration on d 28 (*P* = 0.088). The starch diet resulted in a tendency for a greater number of large follicles on d 0 (*P* = 0.051), and a lower number of medium-sized follicles on d 0 (*P* = 0.004). These data suggest that dietary feed supplements provided 18 d prior to breeding can result in measurable changes in reproductive parameters.

Keywords: Pasture heifers, starch supplementation, fat supplementation, reproduction

List of Abbreviations

ADG	Average daily gain
AI	Artificial Insemination
BCS	Body condition score
BW	Bodyweight
CIDR	Controlled internal progesterone drug release
CL	Corpus luteum
DM	Dry matter
EF	Experimental fat diet
ES	Experimental starch diet
IGF-1	Insulin-like growth factor 1
GnRH	Gonadotropin-releasing hormone
ME	Metabolizable energy
MP	Metabolizable protein
N	Nitrogen
P4	Progesterone
PAG	Placental associated glycoprotein
PGF2α	Prostaglandin F2alpha
TAI	Timed artificial insemination

INTRODUCTION

There is a long history of research focused on improving the reproductive efficiency of beef females (Diskin and Kenny 2014). Accordingly, tools derived from these efforts, such as artificial insemination, embryo transfer, and *in vitro* fertilization, can be leveraged by the beef industry to improve genetic selection efficiency/intensity for enhanced productivity and reproductive efficiency (Rosales-Torres et al., 2017). Adoption of these technologies has been relatively slow, particularly in the United States, meaning their impact on efficiency has also been limited (Dahlen et al., 2014). Producers often cite concerns associated with expense as reasons for limited use of assisted reproductive technologies (NAHMS, 2009). As such, the uptake of these technologies may be more widespread if accompanied by other management recommendations that can help reduce the investment risk.

The efficacy of reproductive technologies is partially reliant on the animal's nutritional status (de Medeiros Bastos et al., 2004). Factors such as a positive energy balance and adequate body condition score are crucial to cattle's reproductive success (D'Occhio et al., 2019). Furthermore, certain feed additives can alter reproductive parameters. For example, the inclusion of omega-3 fats into ruminant diets improved prostaglandin concentrations (Grant et al., 2005). The inclusion of poly unsaturated fatty acids also improves plasma progesterone concentrations and pregnancy rates to timed artificial insemination (TAI) (Brandão et al., 2018; Fontes et al., 2019). Because of the association between nutrition and reproduction, and the poor uptake of assisted reproductive technologies in the U.S. beef industry, the use of feed supplements to improve cattle's reproductive success is a promising low-cost, low-input method to help producers reduce the risk associated with assisted reproductive technologies.

There are numerous mechanisms that influence reproductive performance, one proposed mechanism is the investigation of volatile fatty acids. Volatile fatty acids produced in the rumen, particularly propionate, alter reproductive hormone profiles (Randel and Rhodes, 1980; Rutter et al., 1983; DiCostanzo et al., 1999). Propionate is the primary driver of gluconeogenesis in the ruminant (Huntington, 1990), and may influence reproductive hormone cascades through this gluconeogenic action because improved glucose production is associated with elevated IGF-1 concentrations, which can enhance embryo development (Thatcher et al., 2019) and progesterone production (Spicer and Chamberlain, 1998). In cattle, previous research demonstrated that a jugular propionate infusion increased plasma progesterone (P4) concentrations by d 14 post-ovulation (Bedford et al., 2018). Increased P4 concentrations are associated with improved pregnancy rates (Johnson et al., 1958; Mann et al., 1999; Larson et al., 2007), making propionate a strong candidate metabolite for nutritional modulation of reproduction. However, a dietary strategy to enhance propionate absorption would be needed for increasing propionate supplies to be a viable strategy for modulating reproductive efficiency. Diets greater in starch are widely acknowledged to increase propionate production and plasma propionate concentrations (Bauman et al., 1971), and high starch diets also elevate progesterone half-life in beef cows compared to cows fed a high fiber diet (Lemley et al., 2009). However, the comparison of high starch and high fiber feeds in the previous literature is often confounded with energy level of the feeds. As such, more targeted investigation of how feed supplements with similar energy contents influence reproductive outcomes is warranted.

Supplementing fiber-based diets or supplements with fat may be one method of achieving isoenergetic rations for comparison with starch supplementation. The investigations within the scientific literature regarding how fat can influence reproduction have produced mixed outcomes. Williams and Stanko (2000) supplemented fat and found that there was an increase in the lifespan of the CL and an improved P4 concentration. However, when there was high forage quality available, there was no inherent difference in reproductive performance in animals supplemented with fat (Bellows et al., 2001). As such, additional work is needed to better evaluate how fat supplementation influences reproductive outcomes in energy-sufficient and energy-equivalent scenarios.

Our objective was to evaluate how diets formulated to be isoenergetic and leveraging starch or fat as the primary energy source affected pregnancy rates, follicle growth, placental-associated glycoproteins (PAG's), and growth rate of pasture-grazed heifers. The hypothesis was that heifers supplemented with a high starch supplement would have similar BW and BCS as heifers supplemented with an isoenergetic fat-based supplement but improved reproductive hormone concentrations and greater pregnancy rates.

MATERIALS AND METHODS

All animals sourced in this study belonged to Virginia Polytechnic and State University. All procedures with animals were performed in accordance with the protocols approved by the Institutional Animal and Care and Use Committee in Virginia Polytechnic and State University (IACUC #18-108).

Animals, Diet, and Experimental Design

Twenty-nine predominately Angus heifers (12 MOA and 310 ± 79.8 kg BW) were balanced into two groups according to BW and body condition score (BCS). Groups were randomly assigned to one of two dietary treatments differing in the primary energy source. The experimental fat (EF) diet contained 11.5% of fat and 7.57% starch, and the experimental starch

(ES) diet had 1.03% fat and 47.5% starch (Table 5.1). Concurrent with dietary treatment assignment, heifers were balanced by BW and BCS within a treatment and assigned to one of two different pastures. Pastures were not expected to differ in forage availability, composition, or other relevant characteristics. Rather, the two pastures were necessary to maintain appropriate stocking density for the automated feeders used for supplement delivery. Diets were formulated to meet or exceed the nutrient requirements for beef heifers (NRC, 2016). Additionally, diets were formulated to maintain the same metabolizable protein (MP) and metabolizable energy (ME) concentration. Dietary ingredients and composition for each treatment are included in Table 5.1. Intakes of the supplements were limited by utilizing Greenfeed Smartfeeders (C-Lock, Rapid City, SD). Tall fescue hay, water, and trace mineral salt were offered ad libitum. Heifers were trained to use the feeders from d -22 to -18 relative to d 0 being the day of first artificial insemination (AI) (Figure 5.1).

Estrus Synchronization and Breeding

The ovulation synchronization protocol was based on farm management practices. On d -10, ovulation synchronization began by utilizing the 7 d CO-Synch + CIDR protocol. The synchronization protocol consisted of an insertion of a controlled internal progesterone drug release device (CIDR) (Pfizer Animal Health, New York, NY) and administration of 100 μg of intramuscular gonadotropin-releasing hormone (GnRH) (Factrel; Pfizer Animal Health, New York, NY) on d -10. On d -3, the CIDRs were removed, and 25 mg of intramuscular prostaglandin F2alpha (PGF2α) (PG; Lutalyse; dinoprost tromethamine; Pfizer Animal Health, New York, NY) was given. Heifers were timed-AI (TAI) with semen from 2 bulls at 72 h after CIDR removal concurrent with GnRH administration (d 0; Figure 5.1). Bull selection was based on farm management practices and was balanced across treatments. All heifers were bred at the time of the 2nd GnRH injection, regardless of detecting standing estrus, although heifers observed in estrus were recorded. After breeding, heifers were visually inspected every 12 hours for estrous behavior on d 14 to 24 (Figure 5.1). Heifers displaying standing estrus were rebred 12 h after the 1st heat detection so that the achievement of pregnancy could be maximized. Heat patches were also utilized to aid in estrous detection and to time insemination.

Blood Collection and Hormonal Assays

Blood samples were collected via coccygeal venipuncture into heparinized blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ, USA). After collection, blood samples were immediately placed on ice until transported to the laboratory and then centrifuged at 2,500 x g for 15 min. Blood plasma was frozen at -20 ºC until further analysis. Blood was collected on days -10,1, 2, 3, 4, 5, 6, 7, 10, 14, 18, 21, 24, and 28 relative to the day of first breeding for heifers that did not require rebreeding (Figure 5.1). The heifers that were rebred had blood collected on days 14, 18, 21, 24, and 28 relative to the second breeding day Plasma samples were analyzed for P4 concentrations using a chemiluminescent enzyme immunoassay (1.15% CV; Immulite 2000 XPi platform; Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). On days 24 and 28, relative to the most recent breeding date, circulating placental associated glycoprotein (PAG) concentrations were determined as described previously (Reese et al. 2018). In short, PAG was assessed using an in-house ELISA established by Green et al. (2005) and modified using a polyclonal antibody (Ab 63). Each assay was run in duplicates for each PAG sample (1.1% CV).

Ultrasonography and Ovarian Measurements

Both ovaries were examined using ultrasonography by an experienced technician on days -10, -8, -4, and 0 relative to the first breeding. Ultrasonography was accomplished using an Ibex Evo II (E.I. Medical Imaging, Loveland, CO) with a L7HD transducer utilizing its 5 to 9 MHz linear probe. Both vertical and lateral measurements were taken of all follicles present. To track follicle growth an ovarian map was utilized. In order to not double count or miss a follicle or CL, non follicular reference points were used. These reference points included; ovarian poles, curvature, hilus, and CL if present as described by (Noel et al., 1993). In short, all follicles that were ≥ 5mm in diameter had both vertical and lateral measurements taken. To determine diameter the vertical and lateral lengths were added together and divided by 2. The follicle data were structured based on counts of small (<5 mm), medium (6-9 mm), and large (10-25 mm). Additionally, ultrasonography was used on d 31 for the heifers that did not exhibit heat after first breeding and d 36, 34, 31, and 28 relative to the second breeding date depending on the date they were in estrus and were bred for those heifers that did exhibit standing estrus relative to the first breeding date (Figure 5.1).

Feed Analysis

To best confirm nutrient composition of each supplementary feed, samples were collected once weekly. Hay samples were taken once from every new bale that was put into the field. Feed and hay samples were dried for 24 hr in at 55 ºC to determine dry matter. These samples were then ground with a Model 4 Wiley Mill (A. H. Thomas Scientific, Swedesboro, NJ) and passed through a 1-mm screen. Ash was determined by heating for 8 h in a muffle furnace at 500 ºC. An Ankom 200 fiber analyzer (Ankom Technology, Macedon, NY) was used to determine NDF of the samples with the addition of neutral detergent, heat-stable a-amylase, and sodium sulfite. The same bags used for NDF were subsequently used to determine ADF by utilizing the same Ankom 200 fiber analyzer (Ankom Technology, Macedon, NY) by adding acid detergent. Both NDF and ADF analysis included ash in the sample. Following ADF analysis, the same bags were used to determine the lignin concentration by utilizing acid detergent lignin to determine the sample lignin concentration. Nitrogen (N) was assessed using combustion analysis (Vario El Cube CN analyzer, Elementar Americas Inc., Mount Laurel, NJ). Crude protein was calculated as 6.25 x N. The acetate buffer method of Hall (2009) was used to determine starch by adding α-amylase from *Bacillus licheniformis* (FAA, Ankom Technology, Macedon, NY) and amyloglucosidase from *Aspergillus niger* (E-AMGDF, Megazyme International, Wicklow, Ireland). Lastly, ether extract was determined using an Ankom XT10 extractor (Ankom Technology) with petroleum ether as a solvent (method 920.39; AOAC, 2002). Energy values for the supplements and their diets were estimated from the beef NASEM model (NASEM, 2016).

The calculated nutrient compositions were used collectively with the measured intake of the feed supplement and the estimated pasture intake of OM, CP, NDF, ADF, Lignin, Starch, and EE. The calculated nutrient compositions were then entered into the NASEM (2016) by utilizing the customization of feeds option in the program. The base feeds included fescue hay as our forage, corn grain (ES), or corn gluten meal (EF). The estimated DMI values of both forage and supplemental grain values for each animal were then entered into the NASEM (2016) program to determine the estimated metabolizable energy (ME) and metabolizable protein (MP) intake. These values were analyzed to compare the two treatments in order to see if they calculated with isoenergetic and isonitrogenous intake levels. These calculated values are likely not the actual ME and MP intakes of the animals, however, this method presents a method for ration formulation in practical settings.

Fecal Sampling and Titanium Analysis

Titanium dioxide was added to each concentrate as an inert marker to estimate hay intake. Both concentrate supplements contained 60 g of titanium per 100 kg dry matter (DM) of concentrate. To retrieve and measure titanium in the feces, fecal samples were taken by rectal grab sampling on d 26 to 31, following a similar procedure to Khaitsa et al. (2005). Samples were taken every 6 hours over a 4 d sampling period. The sampling time was rotated forward 1 hour every new day for 4 days to represent a 24 h sampling period fully. Fecal samples were then dried and ground on a Model 4 Wiley Mill (A. H. Thomas Scientific, Swedesboro, NJ) to pass through a 1-mm screen. After grinding, samples were pooled by taking 1 g of each sample to create a single composite sample for the individual animal. The pooled samples were analyzed in triplicate to determine fecal Ti concentrations for each animal. Samples were analyzed by first ashing them into a muffle furnace at 500 ºC for 24 hr. Sulfuric acid was then added to the ash remnant of each sample and boiled for 60 min. Samples were suspended in DI water and allowed to pass through a Whatman 541 grade filter (pore size 22um), and then DI water was added to bring the samples to 50 ml. Samples were analyzed for titanium using inductively coupled plasma atomic emission spectroscopy (Lomer et al., 2000) by Air, Water & Soil Laboratories, Inc. (Richmond, VA).

Statistical Analysis

Analysis was conducted utilizing R version 4.0.3. Data on animal body weights, BCS, ADG, feed intake, energy intake, and nutrient intake were analyzed using a linear mixed-effect model with a fixed effect for treatment and a random effect for pasture. The statistical analysis of PAG was done by using a linear mixed-effect model with a fixed effect for treatment and bull, as well as a random effect for pasture. Progesterone concentrations and follicle size and count were analyzed with day as a repeated measure with the bull and interaction of diet and day as an interaction. The initial condition was used as an additional random effect. Least square means were used for mean separation. Statistical significance was declared at $P<0.05$ and tendencies were defined as $0.05 < P < 0.10$. The first AI conception rate was calculated by dividing the number of confirmed heifers pregnant from the first breeding by the total number of heifers bred. The overall pregnancy rate was calculated by taking the total number of heifers confirmed pregnant and divided by the total number of heifers utilized in the study. Lastly, the total conception rate was calculated by taking the total number of heifers pregnant from both breeding times, and dividing them by all of the heifers that were either confirmed pregnant or exhibited standing estrus and were bred again.

RESULTS AND DISCUSSION

Animal Performance, Nutrient and Energy Intakes

Initial heifer BW and BCS were balanced among treatment groups at the start of the study (Table 5.2). At the conclusion of the study, both the ES and EF supplemented heifers had gained BW; but, the final BW, BCS, ADG, hay intake, and total intake did not differ between the treatments (Table 5.2). The lack of performance differences among animals suggests the dietary treatments provided similar planes of nutrition, despite fairly marked differences in composition. This similarity was purposeful within the experiment because it was our intention to evaluate how feed supplement energy profile, rather than quantity, influenced reproductive outcomes. Based on calculated or predicted values, treatment diets did not result in different intakes of OM, CP, or ME (Table 5.2). However, ES heifers tended to have a greater MP intake, which may have attributed to the numerically elevated growth of ES heifers when compared with EF heifers

(Table 5.2). Although this difference among groups does not meet the statistical cutoff for discussion assigned *a priori*, the fact that it is so close to the cutoff merits discussion. If indeed a true effect, the increased rate of gain in the ES treatment is likely from the greater amount of estimated MP intake in the ES group. Alternatively, the increase in the rate of gain may suggest the diets differed in energy availability in a manner not captured by the NASEM model. When interpreting the reproductive results highlighted within the paper, this caveat is important to consider as Crichton et al (1959) has shown, a greater plane of nutrition can improve reproductive performance. Dietary treatment differences may be associated with improved plane of nutrition associated with the ES diet, rather than exclusively associated with the energy substrate

Follicle Abundance and Size

The number of small follicles did not differ between treatment groups on the days when follicles were measured (Table 5.3). This was similar to the results of medium sized follicles on days -10, -8, and -4. However, on day 0 the EF heifers had a greater number of medium sized follicles compared to the ES heifers (Table 5.3). Additionally, there was no statistical difference in the number of large follicles between treatments on days -10, -8, and -4 (Table 5.3). However, on day 0 the ES heifers trended towards a greater number of large follicles than did the EF heifers (Table 5.3). Of the heifers that exhibited a large follicle on d 0, 10 were from the ES treatment group (66.67%) and only 4 were from the EF group (28.57%). Despite an increased number of large follicles, no significant difference between the maximum follicle diameter between treatment groups on days -10, -8, -4, and 0 were observed (Table 5.3). These results align with previous research that found when dietary starch was increased, the days required for a dominant follicle to reach ≥ 10 mm was decreased, suggesting that a diet greater in dietary starch may improve follicle growth rate (Dyck et al., 2011).

Hormone profiles

The starch supplemented heifers displayed numerically elevated PAG concentration on d 24 and reached a tendency for higher PAG concentration on d 28 (Table 5.4). Conceptually, the greater concentration of PAG makes sense because the starch diet provides a greater quantity of glucogenic precursors. These results align with the “Warburg effect,” which suggests that most of the glucose supplied to the placenta is utilized to provide carbons to the synthesis of nucleotides, amino acids, and lipids for placental development (Vander Heiden et al., 2009). Theoretically, the improvement of function in the placenta would result in an increased concentration of blood plasma PAG’s in the animal. Research on PAG concentrations has shown that they may be excellent predictors of pregnancy in cattle (Thompson et al., 2010). A decrease in PAG concentration has also been associated with an increased incidence of embryonic mortality (Pohler et al., 2016). However, the method of action of PAG is still not well understood. Given the tendency for a response identified in this study, further work is warranted to better explore the relationships among PAG, nutrition, and pregnancy outcomes.

The animal’s initial P4 concentration ($P<0.001$) and time ($P<0.001$) had a significant effect on the concentration of P4. However, diet ($P = 0.715$), bull ($P = 0.238$), and the diet by time interaction ($P = 0.242$) had no effect on the P4 plasma concentration (Figure 5.2). This result is particularly paradoxical because it appears to oppose work previously conducted within our research group where increased post-absorptive supplies of glucogenic precursors increased P4 concentration post-ovulation. There are several differences between these studies which may help explain the opposing responses. For example, the heifers in Bedford et al. (2018) were not bred, they received jugular infused propionate, and they were dairy animals receiving a total-mixed ration. Most-notably, the Bedford et al. (2018) study did not have an isoenergetic control, and jugular infusion of propionate may have more appropriately represented increased dietary energy availability than any propionate-specific responses. Similarly, the present study did not include a hay-only control diet for isolation of energy-supply-related effects. The experimental design specifically assessed how isoenergetic diets differing in presumed energy source altered reproductive metabolism. Although, not statistically different, the numerically elevated progesterone concentrations on the EF diet are sensible, given other studies previously conducted within the literature. For example, Burke et al. (1996) found that ewes supplemented with increased Omega-6 fatty acids exhibited a greater P4 blood plasma concentrations. The fat source used within the EF treatment was soybean oil, which is high in both Omega-3 and Omega-6 fatty acids (Covington 2004). These results are also particularly interesting in reference to the nutrient intake. As stated, the ES heifers did exhibit a significantly higher intake of MP and still did not have a significantly greater concentration of P4 when compared to the EF heifers. There was statistically no difference between the groups, which may outline the energy benefits that the propionate infusion provided toward the P4 differences in our lab's previous work.

Reproductive Performance

No statistical differences in the pregnancy and conception rates between the two treatment groups are presented due to these responses not having repeatability measures. The heifers in the ES treatment group exhibited a numerically greater first AI conception rate than the EF heifers (Table 5.4). Additionally, the ES treatment group showed a slight numerically greater total pregnancy rate, including both the first and second AI rates (Table 5.4). There were 4 heifers that did not show heat after the first AI and were not successfully bred. The ES treatment group had 3 heifers and the EF group had 1 heifer that did not come into heat. Therefore, if those heifers are removed from the calculation of total pregnancy percentage, the ES exhibited a 22% unit greater total conception rate (Table 5.4). The fact that pregnancy rates followed the number of large follicles more so than reproductive hormone concentrations suggests that identifying factors influencing the number of large follicles may be more important for practical strategies to enhance AI efficiency than strategies to modulate endocrinological responses. Perry et al. (2005) found that cows with dominant follicles ≤ 11mm in diameter were associated with decreased pregnancy rates and an increased incidence of late embryonic and fetal mortality with GnRH induced ovulation.

The usage of the 7 d CO-Synch + CIDR protocol as our method to synchronize estrous was due to the typical on farm protocol at Virginia Polytechnic Institute and State University. However, research has found an increase in reproductive performance with the 5 d CO-Synch + CIDR protocol (Whittier et al., 2013). The 5 d CO-Synch + CIDR protocol does require an extra handling period and is more costly due to the extra dosage of PGF2α (Whittier et al., 2013). The objective of this study was to identify a low cost and low input method to implement with the hopes of improving reproductive efficiency. Economic efficiency is also an important factor to consider, as stated previously a primary reason for the slow adoption of assisted reproductive technologies is due to cost (NAHMS, 2009). Therefore, the 7 d CO-Synch + CIDR protocol was the best estrus synchronization protocol for this project.

Irrespective of the direction of these breeding-associated outcomes, the results of this study clearly demonstrate that short-term interventions in dietary nutrient supply pre-breeding can result in significant changes in reproductive hormone concentrations, follicular dynamics, and associated differences in pregnancy outcomes. This rapid physiological response is of particular interest because it may present a low-cost, low-input method to enhance success of assisted reproductive technologies. In many ways, providing a dietary supplement for 21 d pre-breeding may be more feasible and affordable for producers than existing solutions to improve the efficiency of assisted reproductive technologies such as timed-AI, heat checking, or more complex estrus synchronization protocols. That said, there is certainly more research to be done in addition to this study in order to replicate and to refine mechanisms, define responses, and assess consistency. Overall, the comparison of diets suggests that starch-heavy supplements contribute to maturation of large follicles, increased pregnancy rates, and greater PAG concentrations. By comparison, diets with a greater fat content result in elevated progesterone concentrations, retention of medium-sized follicles, and decreased pregnancy rates. Further comparison of energy supply and energy substrate within the same experimental context is needed to contextualize the importance of energy supply and energy source in modulating these various aspects of ruminant reproduction.

DISCLOSURES

The authors declare that no commercial or financial relationships were associated with this research that could be interpreted as a conflict of interest.

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Figure 5. 1. Timeline of timed artificial insemination (TAI) with estrus synchronization. As well as timeline of days that ovarian ultrasonography was performed and progesterone (P4) and placental associated glycoprotein (PAG) blood samples were performed.

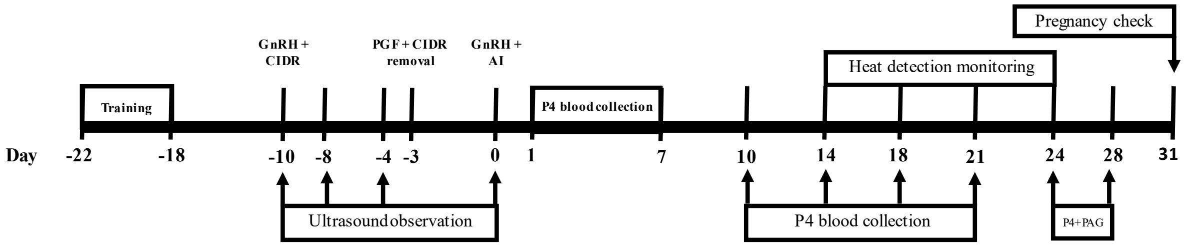

Figure 5. 2. Blood plasma concentrations of progesterone (P4) in beef heifers after breeding. Heifers in the EF treatment group received a supplemental diet high in fat while the ES treatment group received diets high in starch. There was no significant affect by diet (*P* = 0.84) or diet X day (*P* = 0.417) on P4 concentration. Expectedly, there was a significant affect of day on P4 concentration (*P* < 0.001).

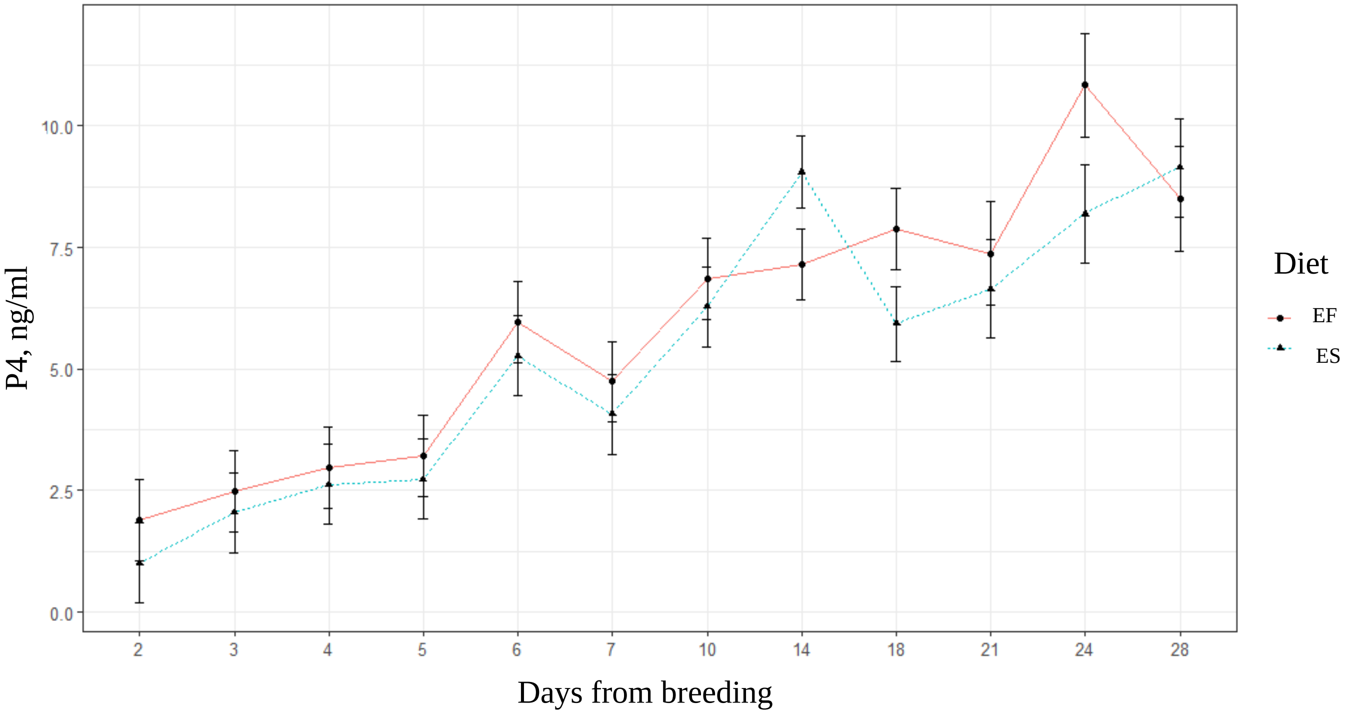

Table 5. 1. Ingredient and nutrient composition of the dietary treatments

	Treatment Diet[1]	
Item	ES	EF
Diet Composition, % of DM		
Grass hay	54.3	53.7
Cracked corn	36.3	-
Soybean oil	-	15.4
Soybean meal	8.2	-
Corn gluten meal	-	28.7
Salt	1.2	2.2
Diet nutrient composition, % of DM[2]		
Starch	47.5	7.57
Fat	1.03	11.53
NDF	42.25	57.54
ADF	19.27	31.41
Lignin	3.12	5.73
OM	94.54	92.81
CP	12.10	10.39

[1] Crossbred Angus-based heifers were randomly assigned to either receive a high starch supplemental diet (ES; n=15) treatment or a high fat supplemental diet (EF; n=14).

[2] Diet nutrient composition was determined through lab feed analysis.

Table 5. 2. Growth performance and intake parameters for beef heifers assigned to two different treatment diets[1]

	ES	EF		
	Mean, kg	Mean, kg	SE	*P*-Value[2]
I-BW[3], kg	310	310	21.2	0.9883
I-BCS[3]	5.37	5.33	0.337	0.872
F-BW[3], kg	364	355	25.6	0.5804
F-BCS[3]	6.46	6.23	0.408	0.4188
ADG[3], kg/d	1.152	0.958	0.178	0.1059
Hay intake, kg	3.98	4.33	0.559	0.640
Grain intake, kg	3.34	3.73	0.586	0.781
Total Intake, kg	7.32	8.06	0.675	0.418
OM Intake, kg	7.47	6.87	0.625	0.491
CP Intake, kg/d	0.837	0.864	0.073	0.782
Starch Intake, kg	0.535	2.97	0.185	<0.001
Fat Intake, kg	0.824	0.084	0.024	<0.001
NDF Intake, kg	3.44	4.79	0.419	0.023
ADF Intake, kg	1.62	2.60	0.215	0.002
Calculated ME Intake, kg	21.1	19.9	1.18	0.440
Calculated MP Intake, g	652	530	48.1	0.0702

[1] Crossbred Angus-based heifers were randomly assigned to either receive a high starch supplemental diet (n=15) treatment or a high fat supplemental diet (n=14).

[2] Statistical significance was declared at $P \leq 0.05$, and statistical tendency was declared at $0.05 < P \leq 0.10$.

[3] I = initial, F = final, BW = body weight, BCS = body condition score, ADG = average daily gain.

Table 5. 3. Follicle parameters measuring the number of differing sized follicles including small (0 – 4.99 mm), medium (5 – 9.99 mm), and large (10 – 25 mm) sized follicles as well as the maximum follicle diameter for pre bred heifers assigned to two different dietary treatments[1]

Day		# Small	*P*-value	# Medium	*P*-value	# Large	*P*-value	Max diameter, mm	*P*-value
-10	ES	3.45	0.539	1.27	0.512	0.392	0.389	9.06	0.336
	EF	4.34		1.04		0.675		7.99	
-8	ES	4.53	0.781	1.45	0.646	0.144	0.807	6.48	0.909
	EF	4.99		1.64		0.17		6.54	
-4	ES	1.99	0.403	1.25	0.607	0.336	0.942	8.8	0.8475
	EF	2.96		1.48		0.348		9.04	
0	ES	1.61	0.8735	0.003	0.002	0.666	0.051	8.2	0.5254
	EF	1.67		0.915		0.291		7.66	

[1] Crossbred Angus-based heifers were randomly assigned to either receive a high starch supplemental diet (n=15) treatment or a high fat supplemental diet (n=14) and follicle measurements were taken on d -10, -8, -4, and 0 with d 0 being the day of first artificial insemination (AI) after being exposed to a 7-day CO-Synch + CIDR estrus synchronization protocol.

[2] Statistical significance was declared at $P \leq 0.05$, and statistical tendency was declared at $0.05 < P \leq 0.10$.

Table 5. 4. Effects of experimental diet treatments on pregnancy rates and placental associated glycoprotein (PAG) concentrations[1]

Variable	ES	EF	SE	*P*-value[2]
First AI conception rate, % (n=29)	53.3 (8/15)	42.9 (6/14)	-	-
Total pregnancy rate[3], %	66.7 (10/15)	57.1 (8/14)	-	-
Total conception rate[4], %	83.3 (10/12)	61.5 (8/13)	-	-
PAG d24[5], ng/ml (n=14)	2.84	1.29	1.54	0.641
PAG d28[6], ng/ml (n=18)	3.84	0.654	2.33	0.088

[1] Crossbred Angus-based heifers were randomly assigned to either receive a high starch supplemental diet (n=15) treatment or a high fat supplemental diet (n=14).

[2] Statistical significance was declared at $P \leq 0.05$, and statistical tendency was declared at $0.05 < P \leq 0.10$.

[3] Total pregnancy rate calculated when all heifers were included in the percentage.

[4] Total conception rate calculated when heifers that did not exhibit second heat before the second breeding were removed from the percentage.

[5] Placental associated glycoprotein (PAG) concentration in heifers that were successfully pregnant on d 24 post breeding, 4 animals were removed due to complications with sample analysis.

[6] Placental associated glycoprotein (PAG) concentration in heifers that were successfully pregnant on d 28 post breeding.

CHAPTER 6

Effect of dietary energy source on reproductive and metabolic hormones, follicle and corpus luteum size and abundance, and granulosa cell gene expression

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List of Abbreviations	
AI	Artificial Insemination
BCS	Body condition score
BW	Bodyweight
CL	Corpus luteum
DM	Dry matter
IGF-1	Insulin-like growth factor 1
GnRH	Gonadotropin-releasing hormone
ME	Metabolizable energy
MP	Metabolizable protein
N	Nitrogen
P4	Progesterone
PGF2α	Prostaglandin F2alpha
TAI	Timed artificial insemination

ABSTRACT

The objective of this study was to evaluate how isoenergetic and isonitrogenous diets, utilizing starch or fat as the primary energy source affected CL size, follicle count and size, progesterone, IGF-1, and IGF binding proteins. Twenty mixed breed Bos-taurus beef cows (715 ± 19.2 kg BW) were assigned to two groups to ensure groups were balanced in terms of BW and BCS. All cows were allowed ad libitum access to pasture forage and water. Animals were assigned to 1 of 2 dietary treatment gain mixes. These grain mixes were delivered through an automated feeder and altered the amount based on initial BW. The experimental diets were formulated to have similar ME (0.97 kg/d) and MP (1.13 kg/d). These diets included one with greater starch (47.5% starch) and the other with greater fat (11.5% fat) diet. Experimental diet feeding began 21 d before a 10 d apart PGF2α pre-synch estrus synchronization protocol and a GnRH injection on d 0. There was also an administration of PGF2α on d 43 and 48 with GnRH provided on d 45. Lastly, there was a follicle ablation performed on d 51 to begin the second follicular wave and an ovum pickup performed on d 60 to collect granulosa samples for gene expression. Cows were followed throughout the 3 estrus cycles, with regular measurement of progesterone, follicle size, and corpus luteum (CL) size There were no treatment differences in final BW, final BCS, forage DMI, supplement DMI, OM intake, NDF intake, ADF intake, ME intake, or MP intake were observed. The starch supplemented heifers exhibited a tendency for greater number of large follicles on d 45 and a greater number of large follicles when the dates that GnRH was administered and the ovum pickup occurred follicle data was combined (*P* = 0.023). The starch diet also exhibited a greater concentration of IGF-1 (*P* = 0.002) and tended to have a lower concentration of P4 on d 43 to 56 (*P* = 0.052). Lastly, there was no observed

difference in granulosa cell gene expression in the genes IGF binding proteins -2, -3, -4, -5, and pregnancy associated plasma protein A.

INTRODUCTION

Optimizing reproductive performance through nutritional management may be an efficient means of enhancing efficacy of assisted reproductive technologies on beef operations. Several studies have inspected the effect of various nutritional supplements on reproductive success when beef females are on a high roughage diet (Bellows et al., 2001; Moriel et al., 2012). Indeed, supplementation to improve or maintain energy balance is undoubtedly critical for successful reproductive efficiency (Funston, 2004). Because supplementation trials often are confounded with dietary energy intake, it is difficult to discern how supplement composition, independent of energy provision, impacts reproduction. In our previous work, increasing provision of energy from starch rather than fat in isoenergetic diets contributed to a greater number of large follicles and numerically improved pregnancy rates (Davis et al. *In Prep*). Although the work demonstrated nutrient composition of supplements impacts reproductive outcomes independent of energy balance, a more mechanistic understand of how these effects are modulated is missing.

Increasing starch content of a diet improves glucose supply both through direct digestive products and through the provision of gluconeogenetic precursor propionate (Bauman et al., 1971). As such, supplementation of starch to the diet may modulate reproductive efficiency through regulation of IGF-1 synthesis, which is sensitive to glucose supplies (Clemmons, 2004)**.** Elevated IGF-1 has a number of positive effects on reproduction, including improving conception rates (Patton et al., 2007), age at first calving (Yilmaz et al., 2006), P4 production (Spicer and Chamberlain, 1998), and embryo development (Velazquez et al., 2005).

Several articles have also linked insulin-like growth factors (IGF) and insulin-like growth factor binding proteins (IGFBP) to follicle dynamics (Voge et al., 2004; Santiago et al., 2005). From these studies, IGF-1 appears to be a significant contributor to the stimulation of mitogenesis and steroidogenesis of granulosa and theca cells in the bovine ovary (Giudice, 2001). IGFBP -2, -4, and -5 fluctuate as the follicle grows, becomes atretic, and ovulates (de la Sota et al., 1996; Stewart et al., 1996). In particular, these three binding proteins decrease as the follicle grows, suggesting reduced IGF-1 bioavailability to the granulosa and theca cells. IGFBP -3 remains relatively constant as the follicle grows, with only minor fluctuations (Funston et al., 1996). Pregnancy-associated plasma protein-A (PAPP-A) is best known for its activity with IGFBP-4 proteases (Rivera and Fortune, 2003). Differences in PAPP-A expression in granulosa cells associated with differences in follicle size have been found in several species, including bovine (Monget et al., 2003; Santiago et al., 2005). However, the nutritional regulation of these genes, within the context of their participation in the IGF-1 signaling cascade, has not been demonstrated.

This study’s objective was to assess the effects of isoenergetic diets differing in starch and fiber content on reproductive hormones, follicular growth, CL growth, and granulosa cell gene expression of mature, non-pregnant beef cows. We hypothesize that the high starch supplemented cows will have improved reproductive hormone concentrations, follicular development, CL growth, and a lower granulosa cell gene expression of IGFBP -2, -3, -4, -5, and a greater expression of PAPP-A. However, we also hypothesize that the high starch diet will have a similar BW and BCS as cows supplemented with an isoenergetic fat-based supplement.

MATERIALS AND METHODS

Virginia Polytechnic and State University owned all animals utilized in this study. All procedures and actions with animals were performed following the Institutional Animal Care and Use Committee's protocols in Virginia Polytechnic and State University (IACUC #20-129).

Animals, Diet, and Experimental Design

Twenty mixed breed Bos-taurus beef cows (715 ± 19.2 kg of body weight BW) were assigned to two groups to ensure groups were balanced in terms of BW and body condition score (BCS). Treatments were then randomly assigned to groups. Treatment diets were designed to create isoenergetic, isonitrogenous diets differing in energy source. Cows were group housed in a single pasture but were individually fed one of two supplements with an automatic feeder (C-Lock, Rapid City, SD). Supplements used corn grain and soybean meal or corn gluten meal and soybean oil as the primary ingredients (Table 6.1). The diet containing the corn grain supplement was designed to provide the majority of its energy through starch (ES), while the diet containing soybean oil was designed to provide the majority of its energy through fat (EF). Much like our previous work (Davis et al., *In Press*), these diets were formulated with the NASEM (2016) software to identical concentrations of metabolizable energy and protein. The purpose of maintaining isonitrogenous, isoenergetic diets within this study was to prevent confounding of energy source with energy quantity; however, the success of this experimental design was partially dependent on the cows consuming pasture at rates similar to that predicted by NASEM (2016). Formulated dietary composition for the two diets is provided in Table 6.1.

The experimental followed a completely randomized design. Animals were fed diets for a 21 day adaptation period, after which they were subjected to an estrus synchronization protocol using two, 12.5-mg injections of PGF2α (Lutalyse HighCon, Zoetis Animal Health) 10 d apart and a 100 μg injection of GnRH (Cystorelin, Merial Canada Inc, Victoriaville, PQ, Canada), and designed to stimulate ovulation. Cows were then followed for 3 estrus cycles, with regular measurement of progesterone, follicle size, and corpus luteum (CL) size. Samples used for gene expression analysis were collected from large follicle granulosa cells on d 60. Fecal samples used to estimate feed intake were collected on d 18 to 22. Details and explanation of each of these procedures are included in the following sections.

Experimental Timeline

In order to achieve the experimental timeline discussed above, careful pharmacological manipulation of the estrus cycle was essential. Synchronization began by providing 12.5 mg of intramuscular PGF2α on d -13 and 3. To achieve ovulation, an administration of 100 μg of intramuscular GnRH was provided on d 0. On d 0 to 10, follicle and CL sizes and quantity were measured via ultrasonography as these days represented the first follicular wave of the reproductive cycle. Also, progesterone was measured on d 0 to 13 to evaluate the rise and concentrations of progesterone between the two treatment groups. Additionally, PGF2α was administered at the same concentration and method on d 43 and 48 with the goal of synchronizing estrus and beginning the first follicular wave. Again, GnRH was provided on d 45 to achieve ovulation and begin the first wave of follicular growth. The size and quantity of follicles were measured on d 45 to 51 to measure follicle growth in the first follicular wave. On d 51, ablation was performed to start the second wave of follicular development. To mitigate pain and distress during the ablation, cows were given an epidural injection of 2% Lidocaine (10-20mL: Butler CO., Columbus, OH). An Aloka 500V ultrasound scanner attached to a transvaginal convex transducer (5MHz) with a needle guide, single lumen 17-gauge 55cm long needle, and a 5 ml sterile syringe manually used for suction was used for the ablation (Aloka, Wallingford, CT). Follicle measurements were taken on d 52 to 60 to measure the size and quantity of follicles in the second follicular wave. Additionally, progesterone was measured on d 43 to 56 to analyze the relative concentrations between the two treatment groups. Lastly, follicular fluid and granulosa cells from the cows showing a dominant follicle were collected via ovum pick-up (OPU) aspiration (n = 17) on d 60 utilizing the same equipment and method previously described with the ablation. After the samples were harvested, they were immediately centrifuged at 2500 RPM for 10 min. Afterward, the follicular fluid was removed from the collection tube and placed into another sterile tube. Once separated, the samples were immediately put into liquid nitrogen until they could be stored at -80 ºC. The samples stayed in the -80 ºC freezer until further analysis was conducted.

Fecal Sampling and Titanium Analysis

Because animals were group-housed on pasture, individual intake of pasture was not directly measured. As such, pasture intake was indirectly estimated based on dilution of an inert TiO_2 marker which was included in the feed at a concentration of 329 mg/kg DM. The procedure for feeding, sampling, and estimation of total DMI from the marker dilution was similar to (Glindemann et al., 2009). In brief, cattle consumed the marker daily in their grain rations for the entirety of the 95 d trial. Fecal samples were collected on days 18 to 22 using a rectal grab sampling technique (Khaitsa et al., 2005). To obtain a composite sample representative of a 24 h period, samples were collected every 6 hours over the 4 d sampling period, with the sampling time rotated forward by 1 hour each day. Fecal samples were frozen immediately after collection until all samples had been obtained. Samples were then dried and ground on a Model 4 Wiley mill (A. H. Thomas Scientific, Swedesboro, NJ) to pass through a 1-mm screen. After grinding, a single composite sample was pooled for each animal by taking 3 g of each individual timepoint sample. The pooled samples were then analyzed in triplicate to determine fecal Ti concentrations for each animal. Samples were analyzed first by ashing in a muffle furnace at 500 ºC for 24 hr. Sulfuric acid was then added to the ash remnant of each sample and boiled for 60 min. Samples were suspended in DI water and allowed to pass through a Whatman 541 grade filter (pore size 22um), and then DI water was again added to bring the samples to 50 ml. The samples were then analyzed using inductively coupled plasma atomic emission spectroscopy (Lomer et al., 2000) at the Virginia Tech Soil Test Laboratory (Blacksburg, VA) to determine exact titanium concentrations.

Feed intake of supplementary feeds was automatically tracked by the feeder used for delivery. In brief, the feeder detects which individual has entered the feeding area using radio frequency identification (RFID). During experimental setup, we programmed the feeders to provide a maximum allowable amount of each supplement based on the initial body weight of each cow, her treatment assignment, and the formulated dietary composition of that treatment. A DMI of 2.0% of body weight was assumed. For example, a 500 kg cow on treatment 1 would be allowed to receive 4 kg of the corn grain-based supplement (500 kg x 0.02 kg feed/kg BW x 0.40 kg supplement/kg feed). The automated feeder would then provide up to that amount of feed to each animal within a 24 h window, while keeping track of the amount of feed dropped to each animal during each feeding bout. To determine pasture intake, the total DMI estimated from the fecal concentrations of Ti was subtracted by the automated feeder measurement of supplementary DMI, and the residual was assumed to be pasture DMI. It is important to note that these supplement DMI measurements were also used in the calculation of total DMI to determine the exact quantity of TiO_2 consumed daily.

Feed Analysis and Calculation of Nutrient and Energy Intakes

To confirm nutrient composition of each supplementary feed, samples were collected once every other week to get the best representation of the feed utilized throughout the study. Feed and forage samples were first dried for 24 hr in a 55 ºC oven to determine the dry matter. Afterward, the samples were ground by using a Model 4 Wiley Mill (A. H. Thomas Scientific, Swedesboro, NJ) and passed through a 1-mm screen. Ash was then determined by heating for 8 h in a muffle furnace at 500 ºC. Neutral detergent fiber (NDF) was measured using an Ankom 200 fiber analyzer (Ankom Technology, Macedon, NY) with the addition of neutral detergent, heat-stable a-amylase, and sodium sulfite. Acid detergent fiber (ADF) was measured in the same bags used for NDF and by using the same Ankom 200 fiber analyzer (Ankom Technology, Macedon, NY) with addition of acid detergent. Following ADF analysis, the bags were used for lignin determination. Nitrogen (N) was assessed by using combustion analysis (Vario El Cube CN analyzer, Elementar Americas Inc., Mount Laurel, NJ). Crude protein was assumed to be 6.25 x N. The acetate buffer method of Hall (2009) was used to determine starch by adding α-amylase from Bacillus licheniformis (FAA, Ankom Technology, Macedon, NY) and amyloglucosidase from Aspergillusniger (E-AMGDF, Megazyme International, Wicklow, Ireland). Lastly, ether extract (EE) was determined by utilizing an Ankom XT10 extractor (Ankom Technology) with petroleum ether as a solvent (method 920.39; AOAC, 2002).

These nutrient composition values were used in conjunction with measured intake of supplement and pasture to calculate intake of OM, CP, NDF, ADF, Starch, and EE. Additionally, the nutrient composition values were entered into the NASEM (2016) program as custom feeds using either fescue hay (forage), corn grain (ES) or corn gluten meal (EF) as base feeds. The DMI values of forage or supplement measured for each animal were then entered into the

NASEM (2016) program individually to determine estimated ME and MP intakes. These values were recorded and analyzed as a means of confirming the formulated isoenergetic, isonitrogenous diets consumed in practice calculated at similar levels of energy and protein as was intended through formulation. Admittedly, calculated ME and MP are not likely to reflect the true ME and MP supplies consumed by the animals in the experiment; however, this represents how rations might be formulated in a practical setting and provides a good guideline for diets of similar energy composition. Furthermore, calorimetric determination of energy consumption was outside the scope of this study and likely would have inhibited our ability to measure the desired reproductive parameters due to changing the production environment of the cattle involved.

Ultrasonography and Ovarian Measurements

To track follicular waves of the cattle on the experiment, both ovaries of each cow were examined using ultrasonography by an experienced technician on days -3 to 0, 1 to 7, 10, and 43 to 60 relative to the first GnRH administration. Ultrasonography was accomplished using an Ibex Evo II (E.I. Medical Imaging, Loveland, CO) with a L7HD transducer utilizing its 5 to 9 MHz linear probe. Both vertical and lateral measurements were taken of all follicles and corpus luteum (CL) present on an ovarian map. In order to not double count or miss a follicle or CL, non follicular reference points were used. These reference points included; ovarian poles, curvature, hilus, and CL if present as described by (Noel et al., 1993). The d 0-10 and 45-51 were of particular interest for first wave follicular growth due to their timing of the estrus synchronization protocol. Additionally, 52 to 60 represented the second wave of follicular growth. The maximum size of the CL was of importance on d 0 to 10 and 49 to 58, as these were the dates when the CL should be developing. Because it is virtually impossible to ensure follicles measured on successive days were the same follicle observed on the previous day, measurements of follicular growth patterns over the timeframes collected were not feasible. As such, follicle data were structured based on counts of small (<5 mm), medium (6-9 mm), and large (10-25 mm) follicles present at key days throughout the protocol (0, 45, and 60). These dates were selected because they best represented the dates when animals would be bred in a time AI situation.

Blood Collection and Hormonal Assays

In addition to tracking follicular patterns, we had an interest in evaluating the reproductive hormone concentrations at key times throughout the protocol. Blood samples for hormone analyses were collected via coccygeal venipuncture. Immediately after collection, blood samples were immediately placed on ice until transported to the laboratory and then centrifuged at 2500 x g for 15 min. The serum was then frozen at -20 ºC until further analysis. Serum was collected for P4 determination on d -21, 0 to 13, and 43 to 60 relative to the first administration of GnRH. Blood was also collected on d 50, 51, 52, 53, 55, and 56 relative to the first administration of GnRH. Analysis of serum P4 and IGF-1 was conducted using Immulite P4 and IGF-1 kits (DPC, Los Angeles, California, USA).

RNA extraction and PCR

To better determine how diet influenced gene expression within the follicular fluid, RNA was isolated from the obtained fluid. To isolate RNA, the QIAGEN RNeasy mini kit (QIAGEN Ltd., West Sussex, UK) was used according to the manufacturer’s instructions. After isolation, RNA was stored at -80 ºC until used in qRT-PCR. Just before use, an aliquot of RNA was thawed on ice for 3 to 5 min. Following the thaw procedure, quantification of total RNA concentration was determined spectrophotometrically (Epoch, Biotech) at the 260 nm wavelength.

Primer and probe design

The primers and probes for qRT-PCR were made by utilizing Primer Express software (Foster City, CA) as previously described (Voge et al., 2004). The bovine sequences for PAPPA-A were found in GenBank and analyzed using the Primer Express software (Foster City, CA) as previously described (Santiago et al., 2005). The bovine sequences for IGFBP -2, -3, -4, and -5 were also found as previously described (Voge et al., 2004). The housekeeping gene utilized was 18s ribosomal subunit = (Voge et al., 2004). Voge et al. (2004) confirmed the efficacy of this housekeeping gene by detecting parallel changes through decreased 18s rRNA amounts and decreasing amounts of the target genes; IGFBP -2, -3, -4, and -5.

Quantitative PCR

The Applied Biosystem High-Capacity cDNA Reverse Transcription Kit (ThermoFischer Scientific; Waltham, MA, USA) and a master cycler were utilized to synthesize cDNA. Afterward, Real-Time reverse transcription-quantitative PCR (Real-Time qRTPCR) was used to quantify relative expression of IGFBP -2, -3, -4, and -5 as well as PAPP-A and the 18s ribosomal housekeeping gene. To test these genes, the wells of an ABI MicroAmp Fast Optical 96-well reaction plates (0.1ml; Fisher 43-469-07, Fisher Scientific) were filled to hold 1 ul of the samples cDNA with the primers for analysis. Once prepped, the plate was read by the Real-Time Fast machine (Applied BiosystemsTM 7500 Real-Time PCR; ThermoFischer Scientific, Waltham, MA, USA). The “Master Mix” contained 5 ul of SYBR Fast Green Master Mix (Fisher 43-856-12, Fisher Scientific), 0.5 ul of both the reverse and forward primers of the target gene, and 3 ul of molecular grade water. The housekeeping gene (ribosomal 18s) was utilized for all qRTPCR analyses and was analyzed in triplicate with each gene as a standard. The five genes were selected because of their known associations with follicular size and growth, and because we were unable to find previously published works which evaluated the impact of diet on expression of these genes.

Data and Statistical Analysis

A generalized linear model was used to analyze the data obtained for initial and final body weights and BCS; intake of feed, nutrients, and energy; and follicular gene expression. The model included a fixed effect for dietary treatment, and least square means were used for comparison among treatments. The number and size of follicles or CL at each measurement point throughout the study were also evaluated using a generalized linear model with a fixed effect for dietary treatment. The progesterone and IGF-1 concentrations were analyzed using a repeated measures approach, with a generalized linear model with fixed effects for initial P4 concentration, day, treatment, and the interaction of day and treatment. Linear orthogonal contrasts were used to compare treatment within day. Significance was declared at $P<0.05$ and tendencies were defined as $0.05 < P < 0.10$.

RESULTS AND DISCUSSION

Animal Performance, Nutrient and Energy Intakes

The treatments appear to have worked as designed due to the overall similarities among animal performance, as well as calculated nutrient and energy intakes (Table 6.2). The initial cow BW and BCS were balanced among treatment groups at the beginning of the study and thus did not differ by design. The final BW and BCS of cows also did not differ between the diets, supporting the idea that animals were consuming similar quantities of nutrients and energy among the diets. This is further supported by the prevailing lack of differences in calculated nutrient or energy intakes; animals did not differ in terms of calculate ME or MP intake among diets. Although calculated intakes are only a proxy for true energy intake, they provide a rough guide to suggest that similar quantities of nutrients and energy were available to the animals irrespective of diet. This is important because numerous studies on dietary effects on reproduction have confounded energy intake and energy source (Ferguson and Chalupa, 1989; Moallem et al., 1999), making it difficult to discern whether differences in reproductive outcomes are due to improved energy status or specific responses to individual nutrient provision.

The notable exception to the generalization that nutrient intakes were similar among studies is related to starch intake, which was greater on the ES diet than the EF diet, by design (Table 6.2). Although supplementary treatments were designed to have greater EE intake on the EF treatment, the calculated intake of EE among diets was not different (Table 6.2). This could have been due to the EF supplement testing relatively low in EE, given its composition. Previous studies using the same supplementary composition (Davis et al., *In Prep*) tested the EF supplement to have EE concentration of greater than 20%. Because the composition was intended to be identical to that used in Davis et al (*In Prep*), this EE result suggests a feed mixing error at the mill or perhaps greater-than-expected variation in the EE content of corn gluten meal between mixed batches of supplementary feed. Another potential explanation could be the lower allowed intake of the EF supplement feed (Table 6.1), which resulted in a significant difference in supplement feed intake among the treatments (Table 6.2). Although the diets were designed with this difference in mind, the lower supplement intake, combined with a lower-than-expected EE undoubtedly contributed to the lack of difference in EE intake among the treatments. Nonetheless, the diets represent isoenergetic provision of diets differing in intake of starch, which allows for determination of how isoenergetic diets differing in energy provision from starch influence reproduction. Thus, this deviation from experimental plan does not negatively impact our ability to test our intended hypothesis.

Hormone profiles

Between d 0 and 13, there was no statistical effect of treatment, day 0 P4 concentration, or treatment by time interaction on P4 concentrations (Figure 6.1). However, there was a significant effect of time, with P4 concentrations increasing from d 0 to 13 ($P < 0.001$). When analyzed between d 43 and 56, P4 concentration was affected by P4 concentration on day 0 ($P <0.001$) and time ($P <0.001$; Figure 6.2); the treatment by time interaction was not significant (P=0.878). The effect of time on P4 was expected due to the natural rise in P4 concentration throughout the estrous cycle (Butcher et al., 1974). Similarly, the association between an animal’s P4 concentrations prior to treatment intervention and the P4 concentrations during treatment intervention suggest strong individual (likely genetic) regulation of P4 concentrations, as well as the stage of the estrous cycle the individual animal is in, which is also supported by previous literature (Veerkamp et al., 1998). Treatment tended to affect the P4 concentrations independent of time (*P*=0.052), suggesting that by d 43 of feeding, dietary treatments had generated consistent differences in P4. The EF diet resulted in greater P4 concentrations than the ES diet. In our previous work (Bedford et al., 2018), differences in P4 concentrations were only observed later in the measurement period, not throughout the entire estrous cycle, as identified here. The differences between these experiments may be dependent on the length of the feeding period. Bedford et al (2018) used shorter feeding periods, meaning that the P4 response may have been establishing while P4 measurements were collected. Because animals in this experiment had been exposed to the dietary treatments for over 40 days prior to sample analysis, it is not surprising that the effect of dietary treatment is more consistent.

It is also logical that the EF treatment group tended to have greater serum P4 concentrations compared with the ES group. These results agree with previous literature which outlines a greater P4 concentration in cattle supplemented with EE (Staples et al., 1998). The dietary fat utilized in this study was soybean oil (Table 6.1). Soybean oil is particularly high in both Omega-3 and Omega-6 fatty acids (Covington, 2004), which have been shown previously to improve circulating P4 concentrations in ruminants (Burke et al., 1996). Previous literature has also highlighted the importance of dietary energy to overall P4 concentration (Gombe and Hansel, 1973); however, the isoenergetic nature of this study implies that the benefit of fat feeding was achieved independent of energy supply. As such, this study agrees with the existing literature on fat supplementation during the estrus cycle.

The CL dynamics were expected to mirror the P4 dynamics; . Although the CL size from d 0 to 10 follows a very similar pattern to the P4 measured during this time period, no significant diet effects on CL size were identified within the study (Figure 6.3). the treatment diets did have a significant effect on serum IGF-1 concentrations Figure 6.4. Treatment significantly (*P* = 0.002) affected, and time tended to affect (*P* = 0.081) serum IGF-1 concentrations. The treatment and time effects were independent and additive, as their interaction was not significant (*P* = 0.985). Overall, the concentration of IGF-1 tended to be elevated on the starch treatment (212 ± 8.4 ng/ml vs 174 ± 8.0 ng/ml). Previous work has also identified elevated IGF-1 concentrations associated with starch feeding (Houseknecht et al., 1988); however, much like the fat and P4 literature, these previous studies were confounded with energy supply. As such, it is interesting to identify that reproductive hormone responses to dietary manipulation are sensitive to nutrient composition without changes in total energy provision. This finding suggests that modulation of reproductive hormone profiles through diet is a fairly sensitive process, responsive to signaling or substrate effects induced by provision of different nutrients rather than driven by wholescale metabolic shifts associated with improved energy balance. The benefits of IGF-1 on follicular growth are well documented (Gong, 2002), and the IGF-1 responses here were reflected within the follicular data, as well be discussed next.

Follicle Performance

Days 0, 45, and 60 were designated *a priori* for statistical analysis of follicle size and number due to the timing of GnRH administration and OPU. Due to the exogenous hormonal modulation of events occurring between these dates, they were viewed as independent and uncorrelated sampling events, rather than repeated measures on the same animal over time. The number of small and medium sized follicles was not different among treatments, irrespective of when the counts were obtained (Table 6.3). The number of large follicles, when averaged over the sampling periods, was significantly affected by treatment, with a greater number observed on the ES diet compared with the EF diet (Table 6.3). The association between ES feeding and development of large follicles is consistent with our work in heifers (Davis et al., *In Prep*). A range of previous work has sought to identify factors which influence follicular maturation. Early in vivo and in vitro studies benchmarked the mechanics of the growth process, and highlighted the importance of reproductive hormones in governing those steps (Wandji et al., 1996; Braw-Tal and Yossefi, 1997). Additionally, in order to best measure the follicular growth rate the days until the dominant follicle reaches ≥ 10 mm were measured. Notably, the ES treatment group exhibited a lower days to reach a dominant follicle ≥ 10 mm in diameter at d 45 – 51 (Table 6.4). More recently, studies specifically evaluating responses to estrus cycle synchronization protocols have highlighted important hormonal differences in cattle ovulating large follicles after GnRH administration (Atkins et al., 2010). In general, our understanding of follicular growth is fairly limited, such that there is even debate about when developmental competency of a follicle is determined (Mermillod et al., 2008). Given the consistency of our findings, and the limited nature of nutritional studies, future research is undoubtedly needed to better understand the role of diet in influencing follicular growth. Specific research questions for these investigations might focus around how long diets must be fed to influence follicle dynamics, the relative impact of different nutrients on follicle growth, and the consistency of findings among breeds, ages, and production settings. Finally, additional work is needed to better understand a possible mechanism for dietary influence on follicle dynamics. Such mechanisms may include direct provision of material essential for cell division, direct signaling pathways, or indirect signaling mediated through hormones or other moderators.

Gene expression

A possible mechanism target evaluated in our work was the indirect modulation through the hormone IGF-1. We hypothesized that gene expression differences in IGF binding proteins and in PAPP-A would be observed across diets, reflecting dietary influence on IGF-1, and its downstream impact on follicle dynamics. Contrary to our expectation, the analysis of gene expression data indicated no statistical differences between the treatment groups for any of the genes measured (Table 6.4). The actions of IGF-1 have been shown to positively influence estradiol synthesis in dominant follicles (Beg et al., 2002), which is an important process in stimulating follicular growth (Stewart et al., 1996). The IGF binding proteins impair the influence of IGF-1 on the follicle because they can bind to IGF-1 and prevent its action (Clemmons, 1993). Pregnancy associated plasma protein A is known for its protease activity on IGFBP-4 (Monget et al., 2003), thus likely supporting the action of IGF-1 by reducing binding. Research has been conducted on these genes in granulosa cells and concluded that IGFBP-2, -3, -4, and -5 are in lower abundance in subordinate follicles as opposed to dominant and larger follicles (Santiago et al., 2005). Additionally, PAPP-A is of lower expression in dominant follicles as opposed to subordinate follicles (Santiago et al., 2005). As mentioned previously, there were greater numbers of large follicles in cows consuming the ES rather than EF supplement (Table 6.3), and elevated IGF-1 concentrations (Figure 6.4). As such, the lack of change in IGFBP and PAPP-A may reflect that dietary-driven modulation of IGF-1 does not trigger changes in follicular gene expression. This may also be reflective of greater regulatory influences being presented by other factors within the hypothalamic-pituitary-gonadal axis or greater reproductive environment. For example, luteinizing hormone has been identified as a potent regulator of gene expression during selection of the dominant follicle (Luo et al., 2011). Another potential explanation may be the difference between follicle development regulation and granulosa cell gene expression. Previous studies have identified a role for insulin in granulosa cell gene expression without observing influence on follicular development (Maffi et al., 2019);

as such, it is plausible that IGF-1 can exert influence on follicular development without exciting changes in granulose cell gene expression. Indeed, in a recent in vitro study, IFG-1 and FSH appeared to activate the LH receptor machinery within oocytes and granulosa cells (Monte et al., 2019), suggesting an alternative pathway for investigating mechanisms whereby diet may influence follicular growth

CONCLUSIONS

Much like our previous work in heifers, this study identified that isoenergetic diets differing in starch content induced important changes in reproductive hormone profiles (P4 and IGF-1), and increased the number of large follicles present prior to ovulation events. These results demonstrate that short-term supplementation can effect on reproductive parameters, and possibly, improve reproductive efficiency. Admittedly, the IGFBP's and PAPP-A gene expression did not provide the results as expected, possibly due to a lack of repetition or because of alternative metabolic strategies for IGF-1 to modulate follicular growth. Further research is necessary to continue to identify feeding strategies, with the goal of improving reproductive success in cattle. The next logical step is to continue to utilize isonitrogenous and isoenergetic diets to investigate further how they can improve conception rates as well as the days to ovulation postpartum.

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FIGURES

Figure 6. 1. Progesterone plasma concentrations (ng/ml) for beef cows assigned to high starch (ES) and high fat (EF) supplementary feedstuffs on d 0 to13. There was no significant affect by diet ($P = 0.803$) or diet X day ($P = 0.609$) on P4 concentration. There was a significant affect of day on P4 concentration ($P < 0.001$).

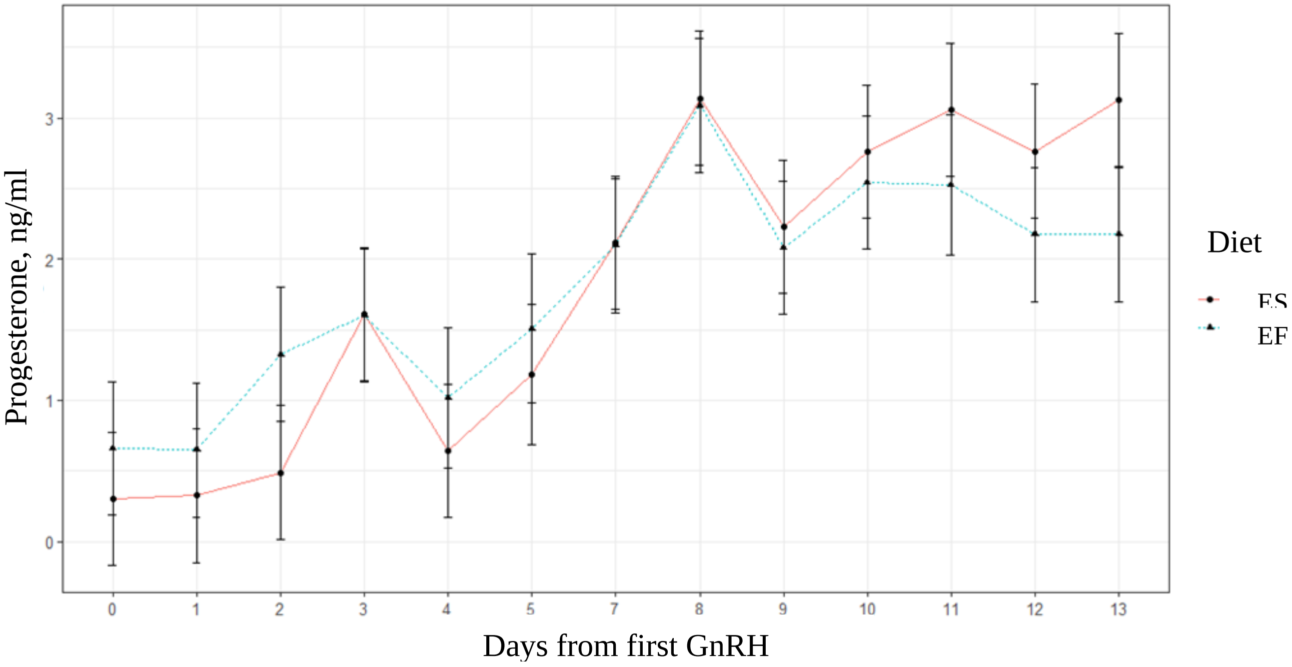


Figure 6. 2. Progesterone plasma concentrations (ng/ml) for beef cows assigned to high starch (ES) and high fat (EF) supplementary feedstuffs on d 43 to 56. There was a significant affect by diet (*P* < 0.001) and day (*P* < 0.001) on P4 concentration. There was not a significant affect of diet X day on P4 concentration (*P* = 0.898).

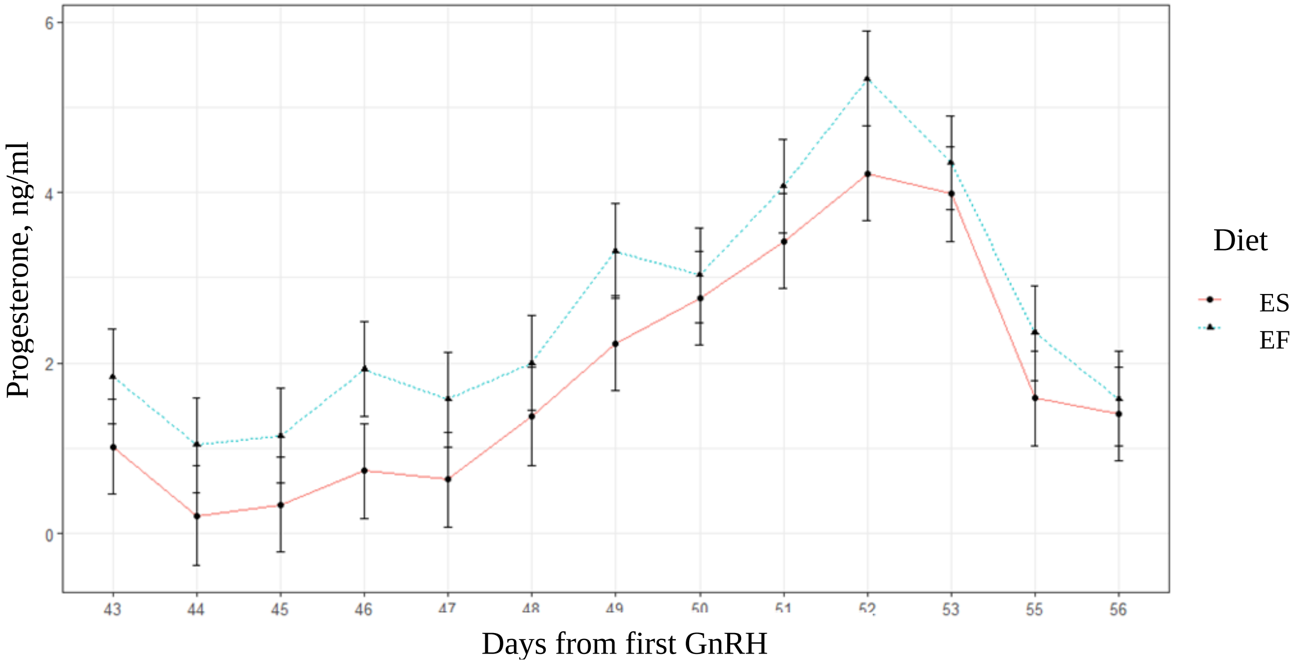


Figure 6. 3. Maximum corpus luteum size (mm) for beef cows assigned to high starch (ES) and high fat (EF) supplementary feedstuffs. There was no significant affect by diet (*P* = 0.375) or diet X day (*P* = 0.197) on maximum CL size. There was a significant affect of day on maximum CL size (*P* < 0.001).

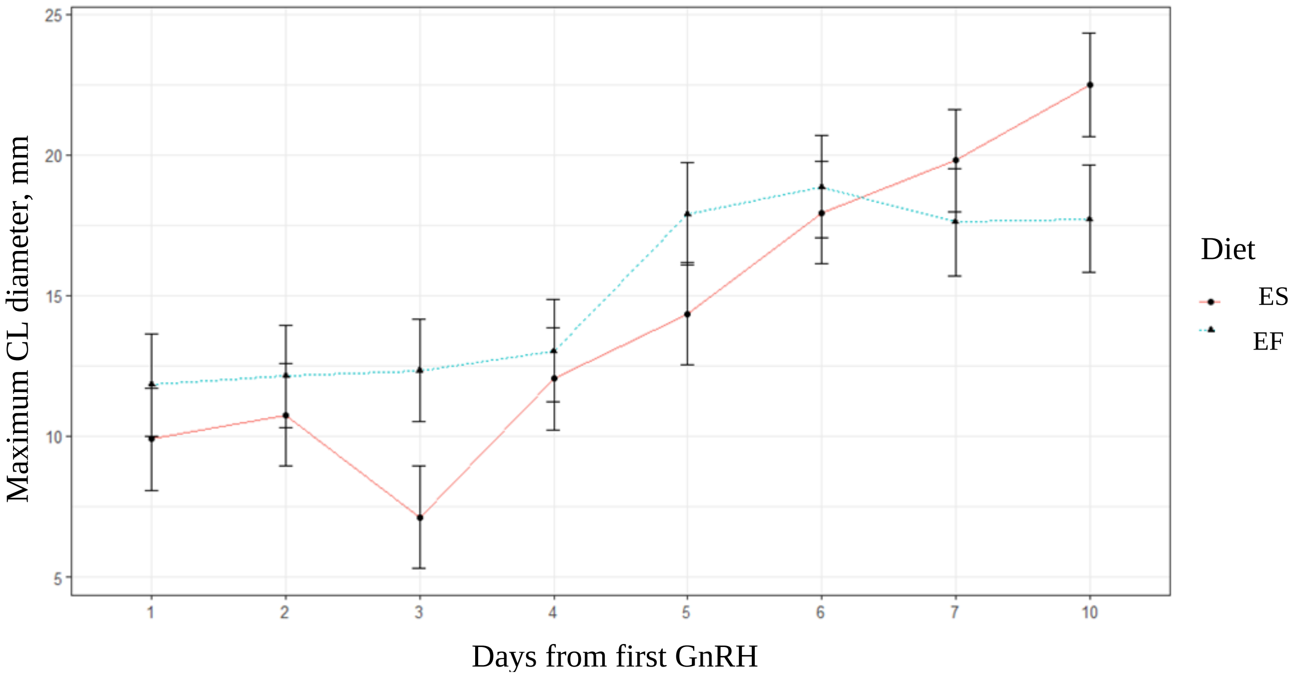
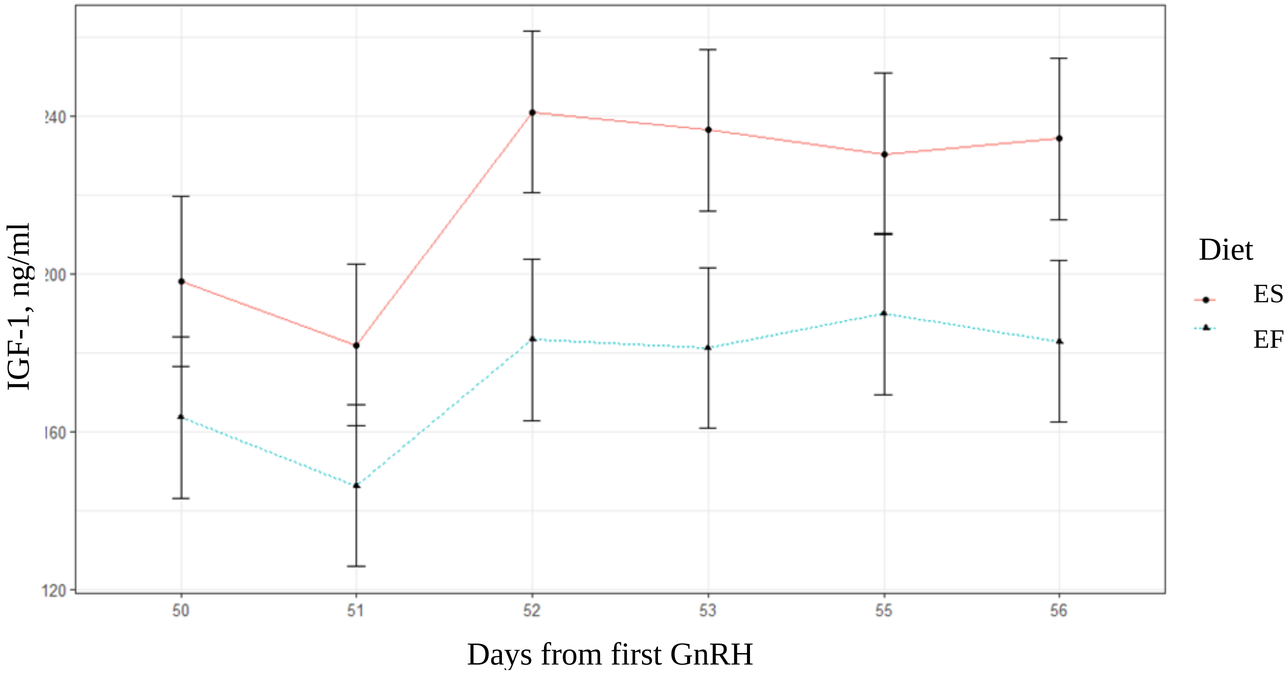


Figure 6. 4. Insulin growth factor 1 plasma concentrations (ng/ml) for beef cows assigned to high starch (ES) and high fat (EF) supplementary feedstuffs. There was a significant affect by diet (*P* < 0.001) and a trend for day (*P* = 0.099) on P4 concentration. There was not a significant affect of diet X day on P4 concentration (*P* = 0.987).



TABLES

Table 6. 1. Formulated dietary feed inclusion rates and supplement nutrient composition for each diet

Item	Treatment Diet[1]	
	ES	EF
Formulated Composition, % of DM		
Pasture grass	60	79
Cracked corn	31	-
Soybean oil	-	7
Soybean meal	7	-
Corn gluten meal	-	13
Salt	1	1
Supplement nutrient composition[2]		
DM, % of as-fed	91.6	88.0
OM, % of DM	94.5	92.8
CP, % of DM	12.1	10.4
NDF, % of DM	41.3	55.3
ADF, % of DM	17.2	28.8
Lignin, % of DM	3.15	5.74
Starch, % of DM	47.5	7.57
Fat, % of DM	2.51	7.46

[1] Crossbred Bos Taurus cows were randomly assigned to either receive a high starch supplemental diet (ES, n=10) treatment or a high fat supplemental diet (EF, n=10).

[2] Pasture consumed during the experiment (DM = 24.3%) was 63.8% neutral detergent fiber (NDF), 29.9% acid detergent fiber (ADF), and 5.75% lignin.

Table 6. 2. Body weight, body composition, and intake parameters for beef cows assigned to high starch (ES) and high fat (EF) supplementary feedstuffs

	ES	EF		
	Mean, kg	Mean, kg	SE	*P*-Value[1]
I-BW[2], kg[3]	724	706	19.2	0.516
I-BCS[2]	6.90	6.85	0.165	0.833
F-BW[2], kg	723	695	19.8	0.338
F-BCS[2]	7.20	7.45	0.136	0.210
Dry Mater Intake, kg	14.2	10.9	2.18	0.295
Pasture intake, kg	11.8	9.17	2.09	0.390
Supplement Intake, kg	2.43	1.71	0.183	0.012
OM[2] Intake, kg	13.5	10.3	2.07	0.287
CP[2] Intake, kg/d	1.74	1.26	0.218	0.136
NDF[2] Intake, kg	8.50	6.80	1.37	0.390
ADF[2] Intake, kg	3.93	3.23	0.644	0.451
Starch Intake, kg	2.09	0.53	0.15	<0.001
Fat Intake, kg	0.325	0.321	0.049	0.955
Calculated ME[2] Intake, kg	30.7	23.2	4.16	0.223
Calculated MP[2] Intake, g	990	715	139	0.178

[1] Statistical significance was declared at $P \leq 0.05$, and statistical tendency was declared at $0.05 < P \leq 0.10$.

[2] I = initial, F = final, BW = body weight, BCS = body condition score, OM = organic matter, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, ME = metabolizable energy, MP = metabolizable protein.

[3] Initial body weights reflect those measured at the midpoint of the experiment due to scale failure at the start of the experiment.

Table 6. 3. Follicle parameters measuring the number of differing sized follicles including small (0 – 5.99 mm), medium (6 – 9.99 mm), and large (10 – 25 mm) sized follicles as well as the maximum follicle diameter for bos taurus beef cows assigned to two different treatments[1]

Day		# Small	*P*-value[2]	# Medium	*P*-value[2]	# Large	*P*-value[2]	Max diameter, mm	*P*-value[2]
0	ES	1.7	0.7763	0.2	0.6278	1.3	0.1069	14.1	0.7178
	EF	2.1		0.3		0.9		13.6	
45	ES	0.9	0.4709	0.7	0.1449	1.4	0.0594	13.9	0.1809
	EF	1.6		0.3		0.8		10.7	
60	ES	0.6	0.8344	1.2	0.3723	1.1	0.5875	13.6	0.4002
	EF	0.8		0.8		0.9		11.6	
All dates	ES	1.07	0.4968	0.7	0.2411	1.267	0.0225	13.9	0.1036
	EF	1.5		0.467		0.867		12.0	

[1] Crossbred Bos Taurus cows were randomly assigned to either receive a high starch supplemental diet (n=10) treatment or a high fat supplemental diet (n=10) and follicle measurements that were deemed a priority were d 0, 45, 60, and all dates combined with d 0 being the day of the first GnRH administration.

[2] Statistical significance was declared at $P \leq 0.05$, and statistical tendency was declared at $0.05 < P \leq 0.10$.

Table 6. 4. Measurement of number of days to reach a follicle diameter ≥ 10mm for bos taurus beef cows assigned to two different treatments[1]

Days	ES	EF	SE	*P*-Value[2]
D 0-10	5.11	5.33	0.342	0.653
D 45-51	3.62	5.2	0.348	0.003
D 52-60	4.22	5	0.375	0.151

[1] Crossbred Bos Taurus cows were randomly assigned to either receive a high starch supplemental diet (n=10) treatment or a high fat supplemental diet (n=10) and follicle measurements that were deemed a priority were d 0, 45, 60, and all dates combined with d 0 being the day of the first GnRH administration.

[2] Statistical significance was declared at $P \leq 0.05$, and statistical tendency was declared at $0.05 < P \leq 0.10$.

GENERAL CONCLUSION

The overarching goal of this work was to investigate different ways that diet can influence reproduction in cattle. Reproductive efficiency is influenced by several factors that need to work in harmony to ensure the highest degree of reproductive efficiency. It is well documented that a positive energy balance is crucial for reproductive function. However, further research was necessary to determine how specific dietary ingredients can influence reproduction in cattle. This work investigates how the entire dietary composition can impact reproductive efficiency in dairy cattle and how a diet known to improve propionate production can affect reproduction in beef cattle. Particularly we demonstrate the influence of the propiogenic diet on pregnancy rates, placental-associated glycoproteins, follicle, and CL dynamics, progesterone concentrations, IGF-1 concentrations, and IGF binding protein gene expression in granulosa cells.

The work described in Chapter 4 focuses on dietary composition, research housing type, dairy breed type, parity, and their relative effects on days to ovulation, pregnancy at first artificial insemination, and overall pregnancy rates in a meta-analysis. This study's objective was to quantitatively summarize the results of dietary composition on cows and heifers' reproductive efficiency. The major conclusions from Chapter 4 are: 1) reproduction is a multifaceted issue where numerous factors influence reproductive performance; 2) a lower days to ovulation is associated with a greater degree of glucogenic precursors and less non-digestible feedstuffs; and 3) a diet high in crude protein, ether extract, non-structural carbohydrates, and low NDF resulted in the highest predicted overall pregnancy rate in multiparous cows.

Chapters 5 and 6 describe two studies that measured the reproductive outcomes from two different diets. One diet involved a starch diet that will have a greater degree of propionate production from the rumen. The other diet involved a fat diet with a low degree of propionate production from the rumen. In both chapters, the diet with a greater degree of fat supplementation showed a significantly greater progesterone concentration. Whereas, the high starch diet showed a greater amount of large follicles at what was and would be the day of breeding in a timed artificial insemination situation. Additionally, the starch diet showed a significantly greater concentration of placental associated glycoproteins in Chapter 5. This work provides further evidence that diet can have a positive influence on reproductive efficiency through dietary composition, rather than just the goal of a positive energy balance.

Overall the present work contributes towards advancing our understanding how diet can influence reproductive efficiency. One novel advancement was the finding that diet tended to have on placental associated glycoprotein concentration in Chapter 5. The implementation of diet to influence reproductive efficiency is a low-cost and low-input method that theoretically will influence the producers profitability and improve animal food products to help feed the world. Additionally, with the use of diet intervention to improve reproductive efficiency, it may aid with the acceptance of assisted reproductive technologies to further improve genetic merit and animal food product production. A second particular advancement of this work was identifying consistent effects of high starch diets on large follicle size relative to the time of breeding in a time artificial insemination situation. Although not statistically evaluated, the improved overall conception rate associated with the starch diet in Chapter 5 is also a noteworthy observation. This information can ideally help with further research identifying ideal supplementation strategies to help with improving reproductive performance in cattle.