SUPPLEMENTATION OF Ω-6 FATTY ACIDS IN COW-CALF OPERATIONS

A Dissertation

by

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ABSTRACT

Four experiments were conducted to evaluate: (**1**) effects of calcium salts of soybean oil (CSSO) supplementation to *Bos taurus* beef cows post-AI on conception rates and (2) on pregnancy establishment factors; (3) effects of CSSO supplementation to late gestating beef cows on the performance of the offspring; (4) the viability of utilizing low-moisture molasses-based blocks (LMB) as a delivery method for CSSO. In Exp. 1, 1,771 cows were divided into groups, and inseminated on d 0. After AI, groups received CSSO (n = 11), or prilled saturated fat (CON; n = 11) from d 0 to 21. Cows receiving CSSO had greater (P = 0.01) pregnancy rates. In Exp. 2, 90 cows housed in 18 pens were assigned to the same treatments and timed AI program from Exp. 1. On d 15, selected cows were assigned to conceptus collection. On d 20 blood was sampled for RNA extraction. CSSO supplementation increased (P = 0.05) mRNA expression of *IFNT* by the conceptus, and blood mRNA expression of ISGs. In Exp. 3, cows were assigned to receive: CSSO (n = 52) or CON (n = 52) during late gestation. CSSO cattle had greater ($P \leq 0.02$) colostrum and plasma IgG; greater ($P \leq 0.05$) expression of adipogenic and myogenic genes; required fewer microbial treatments for BRD (P = 0.05) and had greater LM area compared to CON cohorts. In Exp. 4, 36 cows (n = 9 pens) were assigned to receive: 1) NOSUPP; 2) LMB, 24.7% CSSO; 3) CONC, hand-fed, 24.7% CSSO. Plasma concentrations of linoleic acid, ω-6 PUFA, and total FA were greater ($P < 0.01$) in CONC and LMB vs. NOSUPP cows. Collectively, these results present CSSO supplementation as a strategy to improve reproductive success in *Bos taurus* beef cows and productive performance of offspring born from supplemented dams. These results are associated to effects of linoleic acid and its ω-6 derivates. Additionally, the use of LMB seems to be a valid delivery method for CSSO supplementation, and consequently ω-6 FA, to beef cows.

DEDICATION

This dissertation is dedicated to my family.

My mother, Lucilene Poggi, my sister Leticia Poggi Brandão, my father Fernando Brandão, and my future husband Michael W. Block.

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1. INTRODUCTION

The demand for livestock commodities, including beef, are forecasted to continually increase until 2030 (Robinson and Pozzi, 2011). Hence, strategies to increase beef production and sustainably improve beef cattle efficiency are warranted worldwide.

The utilization of fixed timed artificial insemination (FTAI) of beef cows is a technique utilized by producers to obtain genetically superior and logistically timed calf crops. Despite its proven advantages, implementation of FTAI in cow-calf operations is costly and labor intensive (Rodgers et al., 2012). It is crucial that this technology is associated with management practices to circumvent presented challenges and support its success. Pregnancy loss is a significant challenge for beef cattle operations utilizing FTAI. According to recent comprehensive meta-analysis (Reese et al., 2020), over 50% of all pregnancy losses in beef cattle occur prior to day 16 post-insemination, characterizing early embryonic mortality. Therefore, strategies to reduce embryonic mortality in bovine females are warranted to improve overall efficiency of beef producing systems.

For over a decade, it has been known that supplementation of calcium salts of soybean oil (CSSO) to *Bos indicus* beef cows subjected to FTAI is associated with increased pregnancy rates (Lopes et al., 2009; Lopes et al., 2011) This phenomenon was later associated to the incorporation of ω-6 fatty acids, especially linoleic acid, into reproductive tissues during early gestation (Cooke et al., 2014); which increases the mRNA expression of genes related to maternal recognition (Cipriano et al., 2016) and, consequently, improves pregnancy establishment. This series of studies (Lopes et al., 2009; Lopes et al., 2011; Cooke et al., 2014; Cipriano et al., 2016) was conducted in Brazil, utilizing *Bos indicus* cows consuming tropical grasses. Thus, the validation of CSSO

supplementation as a strategy to improve reproductive success in *Bos taurus* beef cows reared in temperate regions typical of the US warranted further research.

In beef cattle production, the possibilities for improved efficiency are not limited to the early stages of gestation. Research has shown beneficial results in applying concepts of epigenetics to livestock species (Wu et al., 2006; Funston et al., 2010). In beef cattle, different strategies of supplementation during late gestation have shown to improve growth and development of the offspring born from supplemented cowherd when compared to unsupplemented cohorts (Funston and Summers, 2013; Marques et al., 2016b). Among these strategies, is the supplementation of essential fatty acids (Marques et al., 2017). Due to the novelty of the subject, literature on the effects of supplementing polyunsaturated (ω-6 and ω-3) fatty acids to beef cows is still limited. However, Marques et. al (2017) have shown improved performance of beef calves born from dams supplemented with a mixture of ω-6 and ω-3 during the last trimester of gestation compared to counterparts born from unsupplemented dams. Nonetheless, further investigation was needed to unravel the individual roles of ω-6 versus ω-3 fatty acid supplementation, and to elucidate the epigenetic potential of these results.

As evidenced herein, nutritional supplementation strategies for beef dams have often been presented as alternatives to improve overall efficiency of beef production systems (Hess et al., 2008). Despite promising results on the productivity of cow-calf operations (Schauer et al., 2005; Cappellozza et al., 2014; Cooke, 2019), provision of supplemental feed to grazing cattle still challenges beef producers. Consequently, research is also warranted to develop feasibility and efficacy of alternative forms of supplying different forms of feed supplements to grazing beef cows. Self-fed supplements, such as low-moisture molasses-based blocks (LMB) are an option to reduce costs associated with supplementation of forage-fed cattle (Kunkle et al., 2000). This strategy has been extensively utilized for mineral provision to grazing beef cattle (Bowman and

Sowell, 1997; Bailey and Welling, 2007; Ranches et al., 2018). However, there is no description of its use to deliver CSSO as a source of ω-6 FA to forage-fed cows. The manufacturing process of LMB involves extreme temperatures and pH conditions that may interfere with stability of nutrients such as ω-6 FA contained in CSSO (Trater et al., 2003).

Based on the presented, the goal of this work is three-fold: (1) to validate ω-6 fatty acids supplementation as a strategy to increase reproductive success in *Bos taurus* beef cows; (2) to evaluate the effects of ω-6 supplementation to beef cows during late gestation on performance and gene expression of the offspring; (3) to evaluate the utilization of LMB as a delivery method of ω-6 fatty acids to grazing beef cattle.

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2. LITERATURE REVIEW

2.1. Lipids in Ruminant Nutrition

The interest in providing lipids to cattle precedes the knowledge about function and potential effects of different individual fatty acids (Maynard and McCay, 1929; Raper, 1950). Initially, this interest was based mostly in the high energy density of lipids (Chilliard, 1993) and their potential role replacing or being associated with cereal grains in cattle diets. This rationale is particularly important when managing animals with increased energy requirements, such as high producing dairy cows or feedlot cattle (Palmquist, 1984; Huffman et al., 1992).

As different processes of lipid metabolism developed, potential nutraceutical uses of this nutrient became of interest for researchers in animal science (Murphy, 1990; Rustan and Drevon, 2001). Addition of lipids to ruminant diets became more than a strategy to increase energy density, but an alternative to improve performance in specific ways such as interactions with the reproductive and immune systems (Wehrman et al., 1991; Araujo et al., 2010). In studies of human nutrition, light was also being shed on the details of lipid metabolism, with interest growing in different fatty acid profiles of human diets and their potential manipulation to yield healthier food products (Holman, 1998; Tapiero et al., 2002). Alteration of fatty acid profiles of animal products through dietary strategies became of great interest in the fields of animal science (Larick and Turner, 1989; Rego et al., 2005; Partida et al., 2007; Morales-Almaráz et al., 2010; Andreo et al., 2016; Nascimento et al., 2020).

In general, the study of lipid nutrition of livestock species can be divided in 3 different aspects: 1) inclusion of lipids in the diet as a source of energy; 2) inclusion of lipids in the diet due to their different nutraceutical properties; 3) inclusion of lipids in the diet aiming to alter the fatty acid composition of tissues to be harvested as human food. For ruminant nutritionists, however, these

goals pose a much greater challenge than they do to their peers in the pork or poultry industries. The rumen environment limits the use of lipids as a source of energy. Lipids present toxicity to ruminal microbes, reducing digestibility of other diet components, which requires addition of fats and oils to be limited in order to achieve optimal energy efficiency (Hess et al., 2008). This is even more critical for diets with a greater inclusion of forage or fiber due to cellulolytic microbes being more sensitive to the toxic effects of lipids, especially those containing unsaturated fatty acids (Whitney et al., 1999; Brokaw et al., 2001; Buccioni et al., 2012). Thus, addition of supplemental fat as an energy source into ruminant diets must take into consideration its interactions with the ruminal environment. Extensive research has been published studying these interactions, and the overall stablished recommendation is that lipidic content in ruminant diets should not surpass 6% of dry matter intake for high concentrate diets and 2 to 4% dry matter intake for high forage diets, with some variation depending on specific animal, environmental and even commodity market conditions (Hess et al., 2008)

Most of the nutraceutical properties of lipids are related to their FA profile, and particularly to their essential fatty acid (EFA) concentration and composition (Crawford, 1993; Uauy and Dangour, 2006). As it is for other nutrients, the term *essential* designates a compound that cannot be synthesized *de novo* by an organism and must, therefore, be obtained through the diet (Burr and Burr, 1930). Following this rationale, the only two fatty acids that may be considered truly essential for mammals are the linoleic acid (LA; 18:2n-6) and the alpha-linolenic acid (ALA; 18:3n-3), both polyunsaturated fatty acids and classified as ω-6 and ω-3, respectively (Burr et al., 1932). These EFA exert many different physiological functions, interacting with a plethora of signaling, transport and triggering events, some of which will be further discussed herein.

Despite their essentiality, ALA and LA are PUFA and thus, toxic to some rumen microbes, more specifically cellulolytic microbes. As a defense mechanism against PUFA toxicity,

cellulolytic microbes – and among them, particularly *Butyrivibrio fibrisolvens* – can alter the fatty acid composition of the lipid ruminal pool (Kepler et al., 1966; Dehority and Orpin, 1997). Through the process of biohydrogenation, fatty acids with multiple double bonds between carbons may be modified to their most reduced form as a fully saturated fatty acid (Buccioni et al., 2012). For each PUFA, this process includes several isomerase reactions, which yield several isomers as products, which, in turn, may become substrate for the subsequent reaction (Lanier and Corl, 2015). The main consequence of the ruminal biohydrogenation process is a discrepancy between the FA composition of the diet and the FA available for absorption in the small intestine of the ruminant. It has been described that as much as 82 and 86% of all LA and ALA, respectively, contained in a ruminant diet may undergo the biohydrogenation process. Consequently, as little as 18 to 14% of total PUFA fed will be available for the animal for absorption and utilization (Jenkins and Bridges, 2007)

Increasing the amounts of EFA and other PUFA that reach the small intestine of ruminants is a common goal when trying to obtain both the nutraceutical properties offered by these nutrients, and the modification of FA profile of harvested food products. Based on that, methods have been developed to preserve the chemical composition of lipids fed to ruminants and ensure greater absorption of EFA and other PUFA. These methods include practices such as treating unsaturated fatty acids with formaldehydes, or linking them to amines or calcium salts (Jenkins and Bridges, 2007). Some of these methods are utilized in commercial forms of fat supplements, with their main marketable advantage being the increased availability of specific unsaturated fatty acids. Although these protection methods indeed increase the total flow of PUFA to the small intestine of ruminants in most feeding scenarios, none of them is fully prevents ruminal biohydrogenation.

When considering inclusion of lipids in diets of ruminants, the main goal(s) of this nutritional choice must be clearly defined to ensure the appropriate selection of amount, type and overall

feeding scheme of lipid offered. For instance, lipid amount and type included in ration formulated to feedlot cattle will greatly differ from that of a lactating dairy cow diet or of a supplement formulated to be offered to grazing, breeding beef females.

2.2. Lipid Nutrition and Reproduction of the Beef Cow

The main source of revenue of cow-calf operations is the sale of weaned calves, which is economically evaluated based (mostly) on their body weight at weaning (Ramsey et al., 2005). Naturally, strategies to increase these sources of revenue – i.e., ability of beef dams to wean valuable offspring – are of great interest of researchers, producers and other stakeholders within beef producing systems. Reproductive success is strongly related with quantity and quality of the calf crop weaned (Lamb et al., 2016). There is extensive evidence of the relationship between reproductive indicators and number and weight of the offspring produced. This is based on simply having more dams successfully bred, and consequently able to wean a calf by the end of the season. But also, it is associated to other less direct parameters, such as shorter postpartum intervals resulting in earlier conceptions and older calves at weaning (Richards et al., 1986; Michael et al., 2019), as well as adequate response to synchronization protocols and greater conception to AI, which in turn results in an offspring of superior genetic merit compared to natural service sired offspring (Ferraz et al., 2018). Hence, increasing reproductive success of beef cows is crucial to improve efficiency, profitability and, consequently, sustainability of cow-calf operations and overall beef producing systems altogether.

The importance of macronutrients in maternal nutrition of livestock species has been very well stablished for several decades, and in the 1960's and 1970's it was already clear that greater concentrations of energy and protein in cattle diets yielded superior reproductive results (Wiltbank, 1965; Bond and Wiltbank, 1970; Wiltbank, 1970). Although, due to technological constraints of

the time, it was not possible to determine the exact mechanisms causing such results. Lack of tools such as a well established body condition score scale (Wagner et al., 1988), more accurate energy systems or the separation of term '*energy'* into lipidic and carbohydrate component, yielded results often limited in true explanatory value (Wiltbank, 1970) These results, although often inconclusive and speculative, were the foundation for the current knowledge in beef cow nutrition and inspired studies which were then able to address many of the questions left unanswered (Hess et al., 2005).

The first studies that were able to specifically address the relationship between beef cow reproduction and lipid supplementation focused on cholesterol metabolism (Talavera et al., 1985; Williams, 1989; Wehrman et al., 1991). Cholesterol is the precursor of steroids hormones, among them gonadal steroids (such as progesterone, testosterone, and estradiol) that play crucial roles in the endocrine control of reproduction (Hafez and Hafez, 2013). Cholesterol is obtained from lipid metabolism and closely related to dietary fat content (Nestel et al., 1978), which presents an opportunity for nutritional interventions in cholesterol blood levels and subsequent concentrations of reproductive hormones. This rationale was tested, and it was indeed concluded that the dietary lipid content alone, independent of energy density, could alter circulating levels of cholesterol and steroid hormones including progesterone in cycling beef cows (Talavera et al., 1985; Williams, 1989). This conclusion led to further investigation of the relationship between fat inclusion in the diet and other reproductively relevant factors, such as its interaction with different body condition scores (Ryan et al., 1994), follicular dynamics and environment (Wehrman et al., 1991), embryo recovery and viability (Ryan et al., 1992) and effect on anestrous interval of undernourished cows (Ryan et al., 1995).

These studies (Talavera et al., 1985; Williams et al., 1989; Wehrman et al., 1991; Ryan et al., 1992; Ryan et al., 1994; Ryan et al., 1995) followed somewhat consistent treatment models, in which experimental diets consisted of isocaloric and isonitrogenous formulations, differing only

in the lipidic content. Diets with the lower lipidic inclusion, typically around 2% of dry matter intake (DMI), served as control groups, representing traditional feeding schemes of most cow-calf operations. These *control diets* were then compared against isocaloric and isonitrogenous treatments with a high lipid inclusion, ranging from 6 up to 12% of DMI, depending on the study. The rationale of the impacts of lipid supplementation in beef cow reproduction was based on steroid hormone regulation through circulating cholesterol levels (Nestel et al., 1978; Hafez and Hafez, 2013). The correlation of fat supplementation to other metabolic and endocrine controls, such as insulin-glucose status and tissue reserve dynamics, were also considered in some of these studies (Ryan et al., 1994; Ryan et al., 1992), and great progress was made on elucidating the role of lipids in reproduction and general ruminant metabolism.

Because of sole interest in the fat content of diets and due to technological and budgetary limitations of the time, little or no attention was given to the type of fat being fed in those early studies. A variety of lipid-rich feeds such as whole sunflower (Talavera et al., 1985), whole cottonseed (Williams, 1989; Wehrman et al., 1991; Ryan et al., 1995) and soybean oil (Ryan et al., 1992; Ryan et al., 1994) were utilized to formulate treatment diets, with the goal to achieve a high lipid diet that would be consumable and safe to the animals. Therefore, by the late 1990's the reproductive benefits of feeding high fat diets to beef cows were well described and scientifically supported, however, limited knowledge was available on the roles of different types of fat, or fatty acids, on the metabolism and reproduction of beef cattle.

2.3. Ω-6 Fatty Acids and Beef Cow Reproduction

Initial studies investigating effects of fatty acid profiles on beef cow reproduction focused on the comparison of different levels of saturation of dietary lipids (Thomas and Williams, 1996; Thomas et al., 1997b). Among different studies, many aspects prevented data to be applicable from

one work to another, and oftentimes caused discrepancies of expected results and disagreements across conclusions. As example, the roles of linoleic acid in cow reproduction vary greatly according to period of supplementation and amount fed, and specific considerations must be made before concluding on benefit or detriment of reproductive parameters, with enough evidence to support both (Thatcher et al., 1995; Staples et al., 1998; Hess et al., 2005; Hess et al., 2008). In fact, two comprehensive reviews covering impacts of fat supplementation on reproduction of beef cows (Hess et al., 2005; Hess et al., 2008) concluded that feeding linoleic acid to beef cows postpartum was not recommended as a strategy to improve reproductive performance. The overall conclusion was that postpartum supplementation rich in this fatty acid could impair pregnancy rates, compared with lower inclusions of the nutrient (Grant et al., 2003; Grant et al., 2005).

Conversely, for dairy cows, reproductive advantages of diets with greater concentration of PUFA were described more clearly, although there were still reports showing negative impacts associating PUFA supplementation and cow reproduction (Carroll et al., 1990; Sklan et al., 1994). Methodological challenges both in beef and dairy cattle research prevented more clarifying conclusions. Despite extensive data produced, differences in type and manner of fat provision, physiological status of animals and even the small number of experimental units in some of these works, limited the potential for extrapolating results across studies. Thus, the achievement of more holistic and solid conclusions regarding the physiological, reproductive, and financial impacts of fat supplementation to bovine female was hindered, relying heavily on speculations.

Another perceived challenge was the difficulty on compartmentalizing the role of specific fatty acids. Many of the feedstuffs utilized as fat sources in these studies would indeed have a more predominant fatty acid profile regarding ω-3 or ω-6 composition; however, some of these ingredients also presented a mix of both classes of EFA, which may reduce the accuracy of conclusions regarding fatty acid function. In addition, most of this aforementioned research

utilized experimental designs comparing isocaloric, isonitrogenous diets with higher versus lower lipidic inclusion. This approach causes results to be confounding regarding the effects of dietary lipid per se and role of specific fatty acids contained in those lipids. Other researchers compared PUFA to saturated and monounsaturated FA (Thomas and Williams, 1996; Thomas et al., 1997b), which is somewhat more elucidating regarding specific roles of dietary lipids and functions of fatty acids within their classes, and confirmed that not all fat supplements exerted the same effects on reproduction. However, it was slowly becoming clear that the relationship between PUFA supplementation and cattle reproductive physiology was more complex than once thought. The ideas of increased progesterone, or the alterations in the insulin metabolism of animals fed lipid-richer diets were associated with newer and more detailed concepts in reproduction, such as the role of eicosanoids in pregnancy establishment, and more specifically the role of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$).

Eicosanoids are 20 carbon molecules produced in many different systems by a plethora of tissues and cells (Smith, 1989). With functions ranging from protecting the gastric mucosa to mounting a fever reaction, eicosanoids are extremely important to maintain homeostasis and for homeorhetic events such as parturition and inflammation (Higgins, 1985; Shenavai et al., 2012). Eicosanoids are produced from the metabolism of membrane phospholipids and are divided in classes (prostaglandins, leukotrienes) and series (1, 2 and 3), with many different currently known compounds. In a simplified manner, two main fatty acids from the membrane phospholipids, arachidonic acid (AA; ω-6), and eicosapentaenoic acid (EPA; ω-3) are the main precursors for prostaglandins of the series-2 and 3, respectively (Bozza et al., 2011). It has also been generally stablished that, prostaglandins and other eicosanoid originated from AA have a pro-inflammatory character, while those originated from EPA have anti-inflammatory effects (Bagga et al., 2003)

One of the most important eicosanoids in ruminant reproduction is $PGF_{2\alpha}$ (De Rensis and Peters, 1999). Prostaglandin $F_{2\alpha}$ is a series 2 prostaglandin with luteolytic effects and that plays a major role in uterine contraction and involution, being crucial for the process of parturition and the puerperium recovery (Randel et al., 1996). It is synthesized and released in a pulsatile manner by the endometrial cells in response to estradiol and oxytocin and, once in the general circulation, it is rapidly metabolized to 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$. This latter compound is generally referred to as prostaglandin F metabolites (PGFM), which are often the measurable circulating markers for $PGF_{2\alpha}$ (Ginther et al., 2008).

Due to its luteolytic action, PGF_{2a} is highly associated with pregnancy loss, more specifically at early stages of gestation (Knickerbocker et al., 1986). Briefly, if a healthy conceptus is present in the bovine uterus near day 15 day of gestation, it will release interferon-tau (IFN-τ), protein responsible for maternal recognition in ruminants (Bazer and Thatcher, 2017). More specifically, IFN-t silences the estrogen and oxytocin receptors in the endometrium, inhibiting the pulsatile release of $PGF_{2\alpha}$ that causes luteolysis (Thatcher et al., 1997). This way, the process of maternal recognition of pregnancy prevents the luteolytic process to develop, maintaining the corpus luteum and consequently increased concentrations of progesterone necessary for embryo survival. When maternal recognition of pregnancy fails, $PGF_{2\alpha}$ will be released by the endometrial cells and the CL will respond, initiating the process of luteolysis followed by the resumption of an estrus cycle and consequently embryonic death (Lamb et al., 2010).

According to a recent and comprehensive meta-analysis on gestation losses in beef cattle, it was reported that 50% all gestation losses occur prior to day 16 of gestation and, thus, could be associated with failures in the maternal recognition process (Reese et al., 2020). Hence, improving early embryo survival is crucial to enhance reproductive performance of beef females and potentializing the signals involved with maternal recognition of pregnancy may be a valid strategy

to achieve this goal. Decreasing PGF_{2a} concentrations around the pregnancy recognition period has been considered as a possible path to improve pregnancy rates in domestic cattle, and one form to achieve this is through dietary manipulation of the lipidic composition of cell membranes (Thatcher et al., 1995; Staples et al., 1998).

Altering the lipid composition of cell membranes in ruminants via dietary manipulation is a nutritional challenge. However, it has been shown that increasing concentrations of ω-3 PUFAs in the diet is associated to a reduction of PGFM concentrations in cattle, due enzymatic competition with ω-6 PUFAs (Levine and Worth, 1984). This competition was demonstrated in human intestinal cells (Emken et al., 1990). More specifically, the desaturation-elongation pathway, coordinated by Δ-6 desaturase, shows a preference for converting linolenic acid to EPA and DHA, compared to converting linoleic acid to arachidonic acid (Emken et al., 1990). Moreover, rats fed ω-3 FA rich diets showed alteration of the uterine phospholipidic pool, with replacement of ω-6 FA by long-chained ω-3 FA containing 20 and 22 carbons, suggesting a preferential uptake of ω-3 FA by the uterus (Howie et al., 1992).

Fish byproducts, such as fish meal or fish oil, have been largely utilized as sources of ω-3 FA for many species, including cattle (Leaver et al., 1991; Ashes et al., 1992; Olsen et al., 1992; Burke et al., 1997). The main ω-3 fatty acids in fish byproducts are the long-chained EPA and DHA, which have been shown to escape biohydrogenation at greater rate than their ω-3 cohort, linolenic acids (Jenkins, 1993). Fish meal supplementation to lactating dairy cows was associated with increased plasma concentrations of progesterone after a $PGF_{2\alpha}$ injection (Burke et al., 1997). These results were interpreted as a possible lower sensitivity of the CL to $PGF_{2\alpha}$ caused the ω-3FA present in the diet. Thatcher et al. (1997) described decreased plasma concentrations of PGFM after an injection of oxytocin in lactating dairy cows fed fishmeal, compared to control cohorts. This indicates a reduction in the uterine release of $PGF_{2\alpha}$ in response to oxytocin by the cows fed the

fish meal treatment, which had higher plasma concentrations of EPA and DHA compared to control animals. Similarly, beef heifers supplemented with fish meal prior to and during the breeding season had increased conceptions rates and increased plasma concentrations of EPA and DHA compared to control cohorts, which the authors associated to a decreased synthesis of $PGF_{2\alpha}$ (Burns et al., 2002).

In comparison, supplementation of high linoleate – ω-6 FA – feedstuffs has been associated to improved, unaltered, or impaired reproductive performance in cattle. There are reports of decreased or non-different pregnancy rates (Webb et al., 2001; Hess, 2003), and fewer functional CL (Grant et al., 2003) in cows fed diets richer in linoleic acid during the postpartum period. These results have been associated to an increased synthesis and release of $PGF_{2\alpha}$, due to greater availability of linoleic acid, precursor of arachidonic acid, substrate for prostaglandins of the series 2 (Funston, 2004). Indeed, serum concentrations of PGFM were greater in cows fed high-linoleate safflower seeds, compared to other treatment groups receiving diets lower in linoleic acid (Grant et al., 2003). Conversely, other authors have suggested linoleic acid to have inhibitory action on prostaglandin synthase enzymes, thus being potentially associated to reduced $PGF_{2\alpha}$ and improved fertility (Thatcher et al., 1995; Staples et al., 1998; Mattos et al., 2000).

More specifically, Thatcher et al. (1995) described that endometrial microsomes of pregnant cows had greater concentrations of LA and lower concentrations of AA in the free fatty acids pool, which in turn resulted in greater LA/AA ratio when compared to cyclic cohorts at day 17 after estrus. This corresponds to the period of pregnancy recognition for pregnant animals or luteolysis for cyclic animals, when $PGF_{2\alpha}$ is expected to be reduced for the first group and increased for the latter, suggesting a relationship of the FA profile of the tissue and function of the phospholipase enzymes. However, it is crucial to recognize that the changes in circulating PGFM in response to pregnancy recognition events is controlled more than merely by a relationship of availability and

utilization of substrates. At the time of embryonic elongation, concomitantly to pregnancy recognition period, interferon-tau (IFNτ) regulates the differences observed between the pregnant and cyclic uterus including processes controlling the synthesis and release of prostaglandins (Bazer, 1992). Accordingly, Staples et al. (1998) stated that the effects of supplemental dietary fat on cow reproduction are mixed and even though the review focused heavily on dairy cows, references to results in beef cows were made (Williams, 1989; Hightshoe et al., 1991; Wehrman et al., 1991; Ryan et al., 1992; Hawkins et al., 1995; Thomas and Williams, 1996; Wilkins et al., 1996). The rationale for the reproductive benefits observed in PUFA supplemented cows corroborate with the one given by Thatcher et al. (1995), in which linoleic acids is mentioned as precursor of arachidonic acid, but more importantly, as a competitive inhibitor of prostaglandin synthase enzymes and thus likely to be associated to reduction of $PGF_{2\alpha}$ and improved pregnancy rates.

Mattos et al. (2000) supports the same rationale from the previous authors (Thatcher et al., 1995; Staples et al., 1998). However, while Staples et al. (1998) focused on aspects influencing CL lifespan as explanations for the observed reproductive results, Mattos et al. (2000) proposed that there were other controls involved. Alterations in gene expression in the enzymatic apparatus of prostaglandin synthesis and release were suggested by these authors. More specifically, it is speculated that PUFAs exert action upon peroxisome proliferator activated receptors (PPAR) and through these pathways FA altered eicosanoid metabolism (Jump et al., 1996). Or perhaps the action of PUFA is independent of PPARs, acting directly upon gene expression of regulatory enzymes in prostaglandin synthesis such as phospholipase A2 (PLA2) and prostaglandin H synthase (Sessler and Ntambi, 1998). Even though the inhibitory effects of linoleic on prostaglandin synthesis (Thatcher et al., 1995) were mentioned by Mattos et al. (2000), this review

relied mostly on the physiologic role of ω-3 – and not ω-6 – FA as inhibitors of $PGF_{2\alpha}$ synthesis and mainly responsible for the positive reproductive outcomes observed upon fat supplementation.

As previously stated, many of the studies supplementing PUFA to beef cows failed to identify reproductive advantages that justified strategic utilization of fat supplements for a nutraceutical approach (Funston et al., 2004; Hess et al., 2005; Hess et al., 2008). Feeding diets richer in lipids to beef cows seemed to have limited practical advantages, and the physiological effects described years earlier (Talavera et al., 1985; Williams, 1989; Wehrman et al., 1991; Ryan et al., 1992; Ryan et al., 1994; Ryan et al., 1995; Thomas et al., 1997a) often failed to translate to improved reproductive parameters (Funston et al., 2004; Hess et al., 2008). Different outcomes, however, were observed by our research group (Lopes et al., 2009). In a series of 4 large experiments – utilizing 400 to 900 cows per experiment – these authors consistently demonstrated increased conception to timed AI in beef cows supplemented with a rumen-protected PUFA source, predominantly composed of linoleic acid. A fifth experiment in the same publication also demonstrated that PUFA supplementation interacts with progesterone metabolism, not only increasing its synthesis, but also influencing the clearance of the hormone, in a dose dependent manner. Even though the PUFA source utilized, calcium salts of soybean oil (CSSO), had greater concentrations of linoleic acid, it still contained a considerable amount of linolenic acid in its composition as well, preventing a clear conclusion on specific FA role on the observed reproductive outcomes. Another study from our group (Lopes et al., 2011) investigated closely the effects of different periods of CSSO supplementation. It was concluded that the best reproductive outcomes were obtained when cows were supplemented for at least 21 days after AI. No further reproductive benefits were obtained if animals were fed CSSO for 28 days after AI. Also, CSSO supplemented animals performed similarly to control animals in a supplementation period of 14 days. Conjunctly, these outcomes (Lopes et al., 2009; Lopes et al., 2011) allowed authors to

conclude that PUFA supplementation post-breeding exerted effects specifically upon the maternal recognition processes.

To further elucidate FA role and clarify the mode of action of essential fatty acids in beef cow reproduction, our group continued its investigation, now focusing on FA incorporation into reproductive tissues of beef cows fed CSSO (Cooke et al., 2014). This latter study showed that ω-6, and not ω-3 as many other authors have previously claimed, were the main FA responsible for the reproductive improvements recently described (Lopes et al., 2009; Lopes et al., 2011). More specifically, Cooke et al. (2014) reported increased concentrations of linoleic acid and its ω-6 derivates in the plasma, endometrium, corpus luteum and conceptus of cows fed similarly to Lopes et al. (2009) and Lopes et al. (2011). Mechanisms of action were also clarified. Cooke et al. (2014) reported that cows fed CSSO had greater progesterone concentrations and greater CL volume at day 7 post AI compared to control cohorts and higher concentrations of IFNτ in flushed uterine media on day 19 after AI. However, no treatment effects were observed for mRNA expression of genes associated with pregnancy establishment on CL, conceptus, or endometrial tissue on day 19.

In a subsequent study, our group anticipated collection of conceptus and endometrial samples to day 15 of gestation, to better asses the effects of ω-6 FA on expression of genes associated with pregnancy establishment (Cipriano et al., 2016). Results from this work were novel, showing that cows consuming CSSO post-breeding had larger conceptus, which had greater expression of *prostaglandin E synthase* and IFNt day 15 of gestation. No effects on gene expression were observed in endometrial samples, suggesting that the conceptus, more so than the maternal tissues, was the protagonist for the physiological effects observed in the entire series of studies (Lopes et al., 2009; Lopes et al., 2011; Cooke et al., 2014; Cipriano et al., 2016). In fact, when discussing overall results, Lopes et al., (2011) did speculate that a conceptus may be needed to elicit the antiluteolytic effects of CSSO supplementation observed across experiments. When utilizing non-

pregnant cows, Lopes et al., (2011) failed to observe effects of CSSO supplementation on time of luteolysis, incidence of short cycles or progesterone concentrations, which suggested the importance of the conceptus role in the processes involved. Furthermore, the combined results from Cooke et al. (2014) and Cipriano et al. (2016) showed that the reproductive benefits of supplementing a fat source rich in ω-6 were beyond the effects on steroid hormones, luteal lifespan, and eicosanoid synthesis. Cipriano et al. (2016) reported effects of CSSO supplementation on mRNA expression of genes associated with pregnancy establishment in the conceptus (*interferon-tau* and *prostaglandin E synthase*,) and in blood cells of pregnant cows (*interferon-stimulated gene 15*, *myxovirus resistance 2* and *20,50-oligoadenylate synthase*). In contrast, Cooke et al., (2014) failed to report treatment effects on mRNA expression of similar genes. The authors speculated that this was caused by the period when this investigation was conducted, day 19 of gestation, which may have been too delayed for the observation of pregnancy establishment events (Cooke et al., 2014).

Collectively, research from our group suggested ω-6 supplementation, and more specifically linoleic acid, as a strategy to increase pregnancy establishment signals and, thus, improve reproductive performance of beef females. However, all these studies (Lopes et al., 2009; Lopes et al.; 2011; Cooke et al., 2014; Cipriano et al., 2016) were conducted in Brazil, utilizing Nellore cows (*Bos indicus* subspecies) reared in tropical environments. Despite sharing many similarities, the bovine subspecies (*Bos indicus* and *Bos taurus*) also present several differences regarding physiology (Beatty et al., 2006), resiliency (Piper et al., 2009), meat quality (Wheeler et al., 1994) and, more importantly in this case, fetal development (Mercadante et al., 2013; Fontes et al., 2019). More specifically, it has been described that *Bos taurus* cows have shorter gestation length compared to *Bos indicus* (Reynolds et al., 1980) and this is probably due to slightly faster embryonic and/ or fetal growth (Riding et al., 2008; Mercadante et al., 2013). Moreover, tropical

and temperate pastures differ regarding digestibility, protein content and a different forage source may interact with ruminal environment altering the digestion and absorption of rumen protected PUFA (Jayanegara et al., 2011). Thus, further research is warranted to validate the effects of LA and its ω-6 derivates on pregnancy establishment and reproductive success of *Bos taurus* beef cows reared in temperate environments.

2.4. Ω-6 Fatty Fcids and Developmental Programming

Undoubtedly, the number of pregnant females by the end of the breeding season is a valuable and capitalizable parameter for beef producers. However, the input for harvesting a suitable calf goes far beyond a positive pregnancy diagnosis. Harvesting a carcass of high quality is the goal of all meat producing livestock operations, but for beef cattle it is of extreme importance. Beef takes, on average, three times longer to be harvested compared to pork (18 vs. 6 months of age, for cattle and pigs respectively) and 9 times longer compared to chicken, accounting for incubation period (Abdullah et al., 2010; Bonneau and Lebret, 2010; López-Campos et al., 2012). Even though cattle will yield much larger carcasses than pigs or broilers, and consequently deliver more final product, maximizing the productive potential of cattle is crucial for the industry efficiency, profitability, and sustainability. Increasing carcass value of beef animals has been of great interest throughout times, and among the various approaches taken to achieve this goal, maternal nutrition has received increased attention from researchers and other stakeholders in recent years (Funston et al., 2010; Robinson et al., 2013; Mohrhauser et al., 2015).

The concept that dam nutrition affects offspring growth, health, and overall performance later in life can be defined as a form of developmental programming. Initially proposed by the epidemiologist David Barker, the concept of developmental programming, also known as the "*the Barker hypothesis"* was based on the association between premature birth and other pre and peri-

natal characteristics with the occurrence of chronic diseases later in life (Barker and Clark, 1997). Both in humans and animals, the initial interest in developmental programming originated from a preventative perspective. The perceived impacts of maternal nutrition on offspring growth and development were associated to negative effects, such as increased risk for chronic disease in humans and reduced offspring viability and performance in livestock species, when detrimental events occurred during the pre and peri-natal life.

Thus, the pioneer studies of the impacts of in-utero events later in life focused on premature births, low birth weight and stressful events during pregnancy related to health issues that did not manifest until later in life – as late as many decades later (Godfrey and Barker, 2001; Barker, 2002; De Boo and Harding, 2006). This concept is invaluable in human medicine, as it has potential to prevent serious, costly, and dangerous illness, such as coronary heart disease, hypertension, stroke, and diabetes (De Boo and Harding, 2006). In livestock species, however, further and more specific research was warranted to successfully validate this concept due to a much shorter lifespan.

Initial studies with domestic animals focused on nutrient restrictions during pregnancy, which was shown to cause a syndrome generally referred to intrauterine growth retardation (IUGR; Wu et al., 2006). This syndrome does vary depending on the type of restriction and insult imposed on gestating female, and thus manifest itself differently in the produced offspring. Some common and well described expressions of IUGR are late pregnancy abortions, low birth weights, health issues during early life, slower growth compared to herd or littermates (Greenwood et al., 2000; Da Silva et al., 2002; Quiniou et al., 2002). Animals and infants exposed to IUGR may have been referred to as a '*runt'* or one that '*fails to thrive*', popular vague terms that demonstrate an initial lack of full understanding on the causes for different patterns of development among animals or infants apparently exposed to similar conditions (Wolman, 1965; Cooper, 1975).

Although there has been extensive progress in unravelling the biochemical mechanisms involved in IUGR more recently (Jaenisch and Bird, 2003; Wu et al., 2005), they are complex, multifactorial, and dynamic, and still require further investigation for a holistic and applied understanding. Nonetheless, the knowledge acquired thus far is allowing for the development of improved practices, including a more conscientious approach regarding diet formulation and management of pregnant females (Tao and Dahl, 2013; Price et al., 2015; Marques et al., 2016a). These practices were based on the evidence of the long-lasting detrimental impacts of nutrient restrictions in the diet of pregnant females, which are the basis for the IUGR theory (Wu et al., 2006). In contrast, although gross excess of nutrients may also not be advantageous (Pugh and Schumacher, 1993; Ferguson, 2005), there has been evidence of developmental benefits with increasing the concentration of certain nutrients, especially in the manner of micronutrient supplementation – such as trace minerals (Marques et al., 2016a; Harvey et al., 2021a; Harvey et al., 2021b), amino acids (Waterman et al., 2007), and essential fatty acids (Marques et al., 2017; Ricks et al., 2020). In beef cattle production, these approaches expanded to an interest to potentially manipulate marketable phenotypic traits of the offspring, such as muscle growth and immune response (Marques et al., 2016b; Marques et al., 2017; Harvey et al., 2021b). As previously stated, the value of beef cattle is determined by their body weight and composition, with lean muscle being the most valuable characteristic. Thus, ways to improve the production – or growth – of lean muscle in beef animals are warranted. In addition, with growing impositions on antimicrobial usage in livestock species (Paulson et al., 2015; Moran, 2017) strategies to reduce this practice are also of interest and one alternative to achieve this goal is through enhanced immune response to pathogens.

The mechanisms of action involved in the entire process of developmental programming are complex, dynamic (Wu, 2006), and may not always result in measurable differences in phenotypic

traits (Schubach et al., 2019). Briefly, developmental programming consists of epigenetic events, which are defined as genetic, heritable changes such as histone modification and DNA methylation, not involving changes in the DNA sequence (Martienssen et al., 1996). These changes will influence gene expression and silencing, partially responsible for the effects of in-utero interferences observed later in the life of offspring (Paszkowski and Whitham, 2001). Different in-utero events have been shown to have epigenetic effects on the offspring, which has been demonstrated in rodents (McMillen and Robinson, 2005), humans (Murphy et al., 2006) and livestock species such as pigs (Rehfeldt et al., 2004; Wang et al., 2005), horses (Fowden et al., 1994) and cattle (Funston et al., 2010). The type of events also varies greatly in nature. Effects of pre-natal stress of different origins, such as transportation (Price et al., 2015) and thermic (Tao and Dahl, 2013) stressors, have both been shown to impact post-natal development. Dynamics of body reserves also play an important role on fetal and post-natal growth and development of the offspring (Bohnert et al., 2013; Marques et al., 2016b), which is strongly related to energy and protein status of the dams, both shown to be critical controls of growth and development of offspring during the fetal stage, early life and even until many months later.

As scientific methods refine, allowing researchers to perceive more detailed aspects of subjects, it is natural that studies tend to narrow their focus upon those details. In animal nutrition, some of these *details* are the micronutrients. Micronutrients are, by definition, "*elements or substances that are essential in minute amounts to the growth and health of a living organism*" (Dictionary) common examples are trace minerals, vitamins, and more recently EFA (Innis et al., 2013). Even though collaboration of micronutrients to total dry matter intake is practically negligible, their deficiency causes major losses in animal health and productivity, and extensive research has demonstrated consequences of their shortage in cattle nutrition (Cunningham and Loosli, 1954; Smart et al., 1981). Similarly, their dietary inclusion beyond the recommended

requirements (National Academies of Sciences and Medicine, 2016) during of periods high physiological demand, such as gestation (Marques et al., 2016a; Marques et al., 2017) and early lactation for cows (Uchida et al., 2001), as well as post-weaning and upon feedlot entry for growing cattle (Araujo et al., 2010; Lippolis et al., 2017) has shown potential productive benefits. Hence, novel approaches to developmental programming in beef cattle have been focusing on the epigenetic potential of micronutrients, such as trace minerals (Marques et al., 2016; Harvey et al., 2021a; Harvey et al., 2021b) and essential or polyunsaturated FA (Marques et al., 2017; Schubach et al., 2019). The epigenetic role of essential fatty acids has been extensively demonstrated, especially in epidemiologic studies with humans or utilizing rodent models (Shrestha et al., 2020)

Similarly to what has been reported in early pregnancy (Cipriano et al., 2016), there is evidence to support the rationale that ω-6 FA have modulating effects on gene expression in the fetus during later stages of pregnancy and these effects may manifest phenotypically later in life (Marques et al., 2017). More specifically, it is proposed that LA acts upon both adipogenic and myogenic pathways during pre-natal life. Briefly, there is evidence that LA increases the expression of *peroxisome proliferator-activated receptor gamma* (PPARγ), which regulates other adipogenic genes and ultimately increases adipocyte populations, being a genetic marker for adiposity and marbling in beef cattle (Houseknecht et al., 2002; Lim et al., 2011). In the myogenic pathway, a possible mode of action is through increased expression of regulatory factors such as *myogenic differentiation 1* (MyoD) and *myogenin*, which are related to differentiation of satellite cells into muscle fibers, leading to increased number of muscle fiber, ultimately resulting in potential for greater lean muscle mass, as the final muscularity will depend upon not only cellular hyperplasia, but also hypertrophy (Du et al., 2010).

Marques et al. (2017) reported increased muscle growth and adiposity in calves whose mothers were supplemented with rumen protected PUFA (a mix of ω-3 and ω-6 FA). More specifically,

cattle born from dams receiving a supplement rich in LA, EPA and DHA during late gestation showed greater average daily gain during growing and finishing phases, greater body weights and hot carcass weights, greater marbling, and consequently greater percentage of Choice carcasses compared to control cohorts whose dams received an isocaloric, isonitrogenous and isolipidic supplement rich in saturated FA. These authors attributed the observed treatment differences to programing effects of PUFA supplementation during late gestation, however, no genetic markers, such as mRNA expression of targeted genes or DNA methylation, were evaluated. Schubach et al (2019) reported no phenotypical differences on young beef calves supplemented with ω-6 FA, but evidence of developmental effects. More specifically, these authors supplemented nursing calves from 2 to 4 months of age with isocaloric, isonitrogenous and isolipidic treatments differing in the FA profile, which were either rich in n-6 FA or saturated FA. No treatment differences on animal performance were reported throughout the experiment; however, mRNA expression of adipogenic genes – *fatty acid binding protein 4* (FBP4), *fatty acid synthase* (FASN) *stearoyl coA desaturase* (SCD) and PPARγ were increased when animals were 14 months of age suggesting programming effects of ω-6 supplementation.

Collectively, the outcomes reported by Marques et al. (2017) and Schubach et al. (2019) strongly suggest that the supplementation of LA during periods of developmental plasticity may improve muscle growth – translated as greater carcass weights – and adipogenicity – translated as marbling – of the offspring. However, literature investigating the modes of action of programming effects of LA and other ω-6 in beef cattle are still very limited and warrant further investigation, especially considering that Marques et al. (2017) utilized a mixture of ω-3 and ω-6 FA, which may confound the roles of different FA in the epigenetic pathways.

Additionally, supplementing pregnant beef cows with ω-6 FA may impact offspring health. Marques et al. (2017) reported increased plasma haptoglobin concentrations in calves born from

control dams when compared to their PUFA cohorts. Haptoglobin is an acute phase protein synthesized and released in the blood circulation by liver in response to inflammation (Ceciliani et al., 2012) and stress (Cooke and Bohnert, 2011) in cattle. The greater haptoglobin plasma concentration observed in the control offspring at weaning is indicative of heightened stress and inflammation reactions, which could be associated to an impaired immune response (Cooke, 2017). However, Marques et al. (2017) failed to report any treatment differences on the occurrence of BRD or on the administration of antimicrobials from weaning to slaughter. Perhaps the low incidence of the disease in this study, less than 7%, (Snowder et al., 2006) hindered more elucidating results.

More recently, supplementation of ω-6 FA to beef cows during late-gestation has been shown to improve colostrum quality (Ricks et al., 2020). More specifically, the authors reported greater IgG concentration in the colostrum of cows supplemented with ω-6 FA compared to control animals. As a result, offspring born from ω-6 FA supplemented dams had greater serum IgG concentrations at 24 h and 5 days after birth, compared to calves born from control cows. Concentrations of IgG 24h after birth have been shown to have life-long effects on cattle health (Wittum and Perino, 1995). However, this study (Ricks et al., 2020) followed calf performance only until weaning and did not evaluate incidence of economically important diseases later in life, such as BRD during the feedlot stage.

In conclusion, evidence indicates positive productive outcomes of ω-6 FA supplementation to late-gestating beef cows. However, further research is warranted to elucidate the specific roles of ω-6 FA, in comparison to their association with ω-3 FA (Marques et al., 2017). Furthermore, epigenetic mechanisms through which ω-6 FA exert action perceived as phenotypic differences are also still unclear. Finally, an evaluation of lifelong effects of ω-6 FA supplementation on the

health of the offspring is also necessary to support and expand on current findings (Ricks et al., 2020).

2.5. Supplementation Strategies for Grazing Cattle

Beef cows are often reared in extensive grazing systems without access to a complete diet (Bohnert and Stephenson, 2016; Do Carmo et al., 2016). In many operations around the world, beef cows will be offered simple formulations, not more than a few ingredients, to supplement the forage source. These ingredients are often chosen based on practicality, cost of commodities available to beef producers and energy and protein content (DelCurto et al., 2000; Larson et al., 2009). Information on the lipid composition of livestock supplements, when available, is generally limited to ether extract or crude fat content. Thus, the entire concept of supplementing ω-6 FA to beef cows to obtain the reproductive and productive benefits described herein is of difficult application. Many of the research studies cited in this work (Lopes et al., 2009; Lopes et al., 2011; Cooke et al., 2014; Cipriano et al., 2016; Marques et al., 2017; Schubach et al., 2019) utilized commercially available forms of protected PUFA for cattle supplementation. This somewhat facilitates the provision of essential fatty acids to beef cows, as these products are consistent in the amount and composition of specific FA, compared to other lipidic sources such as oils, whole seeds, or by-products.

Besides, nutritional supplementation to grazing animals, especially in larger operations, inputs considerable costs associated with labor, feed transport and storage (Miller et al., 2002), which may discourage producers to supplement beef cows. Reduction of these inputs is an important initial step towards producers' adoption of ω-6 FA supplementation to gestating beef cows, which has been shown to potentially increase reproductive (Lopes et al., 2009) and productive (Marques et al., 2017) performances in beef operations. Free-choice, or self-fed

supplements are a strategy to reduce feeding costs associated with labor, transport and storage of feedstuffs (Kunkle et al., 2000). Methods of free-choice and self-fed supplements have been extensively and successfully utilized for supply of mineral for grazing beef animals (Bowman and Sowell, 1997; Bailey and Welling, 2007; Ranches et al., 2018). Due to their chemical nature, low in organic matter, mineral supplements present lower risk of spoilage under the weather and of over-consumption by the animals. Besides, formulations are available to further lower these risks and ensure safety of these products (Kunkle et al., 2000). Provision of energy and protein rich supplements *ad libitum* requires more intense manipulations to achieve similar levels of safety and economic feasibility, such as the utilization of low-moisture molasses-based blocks (LMB) (Moriel et al., 2019). Briefly, to ensure the proper consistency and stability of LMB their manufacturing process involves changes in pH and temperatures, which may interfere with availability and digestibility of nutrients, such as PUFA (Trater et al., 2003; Katulski et al., 2017). Compared to hand-fed supplements, LMB also present greater variations in intake (Bowman and Sowell, 1997). Inconsistent intake patterns can also influence absorption and digestion of nutrients (Beaty et al., 1994; Farmer et al., 2001; Moriel et al., 2012) and it is unknown if and how these alterations would impact PUFA tissue availability and, consequently, the overall benefits of their supplementation.

Hence, utilization of *ad libitum* supplementation methods, such as LMB, to provide ω-6 FA to forage-fed beef cows warrants scientific validation. This concerns especially the effects of intake variation on PUFA digestion and absorption and PUFA integrity when submitted to extreme manufacturing conditions. Providing an efficacious alternative of supplementing ω-6 FA that reduces the inputs of the feeding process is an efficient mean of advancing the adoption of such technology by cow-calf producers.

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3. SUPPLEMENTING CA SALTS OF SOYBEAN OIL AFTER ARTIFICIAL INSEMINATION INCREASES PREGNANCY SUCCESS IN *BOS TAURUS* BEEF COWS*

3.1. Introduction

Early embryonic mortality is a major reproductive challenge in cow-calf systems and is defined as losses that occur from fertilization to d 27 of gestation (Humbolt, 2001). Strategies to enhance early embryonic survival are thus warranted for optimal reproductive and overall efficiency of cow-calf operations. Our research group reported that supplementation with Ca salts of soybean oil (CSSO) for 21 d beginning after artificial insemination (AI) increased pregnancy rates by 30% in *Bos indicus* beef cows (Lopes et al., 2009; Lopes et al., 2011). This outcome was credited to enhanced early pregnancy maintenance (Spencer and Bazer, 2004), and later associated with incorporation of linoleic acid and its ω-6 derivatives into maternal and embryonic tissues (Cooke et al., 2014). Complementing these findings, Cipriano et al. (2016) reported that CSSO supplementation to *B. indicus* beef cows increased conceptus growth and mRNA expression of *interferon-tau* (IFNt) and *prostaglandin E synthase* on d 15 of gestation, which are critical regulators of pregnancy establishment in cattle (Spencer and Bazer, 2004; Erdem and Guzeloglu, 2010; Dorniak et al. 2011).

Collectively, these outcomes provided evidence that supplementing CSSO to beef cows after timed AI enhances pregnancy establishment by increasing conceptus development and signaling via the IFNt cascade (Cipriano et al., 2016), whereas these effects are modulated by ω-6 fatty acids (**FA**; Cooke et al., 2014) and result in increased pregnancy rates to AI (Lopes et al., 2009, 2011). In contrast, the previously cited experiments were conducted with *B. indicus* cattle reared in tropical environments. Pregnancy establishment and overall reproductive physiology differ among *B. indicus* and *B. taurus* females (Carvalho et al. 2008; Mercadante et al., 2013), and FA composition

differ among tropical and temperate feed ingredients. Hence, research is warranted to validate these outcomes in *B. taurus* cattle in typical U.S. operations. Based on this rationale, we hypothesized that CSSO supplementation after timed AI would increase ω-6 FA intake and absorption, favor embryonic responses required for pregnancy establishment including the IFNt-signaling cascade, and increase pregnancy rates to AI in *B. taurus* beef cows. To test this hypothesis, Exp. 1 compared pregnancy rates to timed AI whereas Exp. 2 compared hormonal, uterine, and conceptus factors associated with pregnancy establishment in *B. taurus* beef cows supplemented or not with CSSO for 21 d after timed AI.

3.2. Materials and Methods

All animals were managed in accordance with the practices outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010; Exp. 1), and experimental protocols reviewed and approved by the Oregon State University Institutional Animal Care and Use Committee (Exp. 2; #4938).

3.2.1. *Experiment 1*

Animals, treatments, and sampling. This experiment (d -10 to 55) was conducted on cow-calf operations (n = 7) managed by the Virginia Department of Corrections, with a total of 771 suckled, lactating, multiparous, non-pregnant Angus cows [mean ± SE; age = 5.98 ± 0.11 yr, days postpartum = 65.2 ± 0.6 d, and body condition score (BCS) = 5.21 ± 0.03 according to Wagner et al., 1988]. Across locations, cows were ranked by BCS and days postpartum on d -10, and allocated to a total of 22 groups averaging 35 cows each (range = 22 to 50 cows/group) in a manner that average BCS and days postpartum were equivalent among groups. Groups were maintained in individual tall fescue-dominated pastures (*Festuca arundinacea*) with ad libitum access to forage, mineral supplement, and water throughout the experimental period. From d -10 to -1, groups were supplemented daily with (as-fed basis) 100 g of ground corn + 100 g of soybean meal

per cow. Supplements were provided in feed bunks placed within each pasture (1.0 m/cow of linear bunk space), and readily consumed by cows within 15 min of feeding.

Groups were enrolled in an estrus-synchronization + fixed-time AI protocol (Larson et al., 2006) from d -10 to 0. More specifically, cows received 100 µg of gonadotropin-releasing hormone (Factrel; Zoetis, Florham Park, NJ, USA) plus a controlled internal device release (CIDR) containing 1.38 g of progesterone (P4; Zoetis) on d -10, 25 mg of prostaglandin $F_{2\alpha}$ (Lutalyse; Zoetis) and CIDR removal on d -3, followed in 60 h by a second 100 µg injection of gonadotropin-releasing hormone and AI (d 0). Multiple AI technicians (n = 11) and semen from different *B. taurus* sires (n = 13) were used across locations and groups, but balanced between treatments within each location. Estrus detection patches (Estrotect; Rockway Inc., Spring Valley, WI) were applied on d -3 to all cows, and occurrence of estrus was recorded at timed AI. Estrus was defined as removal of > 50% of the rub-off coating on the patch (Thomas et al., 2014). Immediately after AI, groups within each location were assigned randomly to receive (as-fed basis) 100 g of ground corn + 100 g of soybean meal per cow daily, in addition to: 1) 100 g/cow daily of CSSO (Essentiom; Church and Dwight Co., Inc., Princeton, NJ; n = 11), or 2) 87 g/cow daily of prilled saturated fat (EnergyBooster; Milk Specialties, Eden Prairie, MN) + 13 g/cow daily of limestone (CON, n = 11). Treatments were formulated to be isocaloric, isonitrogenous and isolipidic but differing in FA composition, and offered from d 0 to 21 in the same feed bunks used from d -10 to -1 (Tables 3.1 and 3.2). Limestone was added to CON to balance treatment Ca content (Table 3.2). Cows consumed treatments by 15 min after feeding, which prevented intake of treatments by calves. Cows were exposed to natural service ≥ 10 d after timed AI. Cow BCS (Wagner et al., 1988) and pregnancy rates to timed AI, assessed with transrectal ultrasonography by the presence of a viable fetus (5.0 MHz linear transducer, Ibex Pro, E.I. Medical Imaging, Loveland, CO), were determined between d 45 to 55 after AI. Pregnancy rates to bull breeding

were beyond the scope of this project and thus not evaluated, whereas potential intake of supplements by bulls were accounted for by increasing treatment offer by 900 g (equaling 3 cows) per group.

3.2.2. *Experiment 2*

Animals and treatments. This experiment (d -10 to 30) was conducted at the Oregon State University – Eastern Oregon Agricultural Research Center (Union station), with 90 suckled, lactating, multiparous, non-pregnant Angus × Hereford cows (mean ± SE; age = 6.81 ± 0.26 yr, days postpartum = 63.6 ± 1.2 d, body weight = 572.5 ± 5.8 kg, and BCS = 5.11 ± 0.04 according to Wagner et al., 1988). Cows were ranked by BCS and days post-partum, and allocated to 18 pens (5 cows/pen) in a manner that pens had equivalent BCS and days post-partum at the beginning of the experiment (d -10). To facilitate cattle management and sampling procedures, pens were divided randomly into 2 groups (group A = 10 pens, group B = 8 pens). Groups started the experiment over 2 consecutive days following the same experimental schedule (d -10 to 30). Throughout the experimental period, cows were maintained in drylot pens (450 m^2, with 2.0 m/cow of linear bunk space) with their respective calves, receiving 20 kg/cow daily (dry matter basis) of grass-alfalfa and ad libitum access to water and mineral mix.

From d -10 to -1, cows within pens were supplemented with (as-fed basis) 100 g of ground corn + 100 g of soybean meal per cow daily, which was offered separate from hay and readily consumed by cows within 15 min of feeding. On d -10, cows from all pens were enrolled in the same estrus-synchronization + fixed-time AI protocol (Larson et al., 2006) described in Exp. 1, including the use of estrus detection aids (Estrotect; Rockway Inc.). All cows were inseminated on d 0 by the same technician, using semen from the same Angus bull and batch. Immediately after AI, pens were assigned randomly to receive the same treatments described in Exp. 1 (n = 9 pens/treatment, with 5 pens/treatment in group A and 4 pens/treatment in group B). Treatments

were offered from d 0 to 21 in the same feed bunks used from d -10 to -1, and cows consumed treatments by 15 min after feeding, which prevented intake of treatments by calves as in Exp. 1.

Sampling. Samples of hay and supplement ingredients were collected before the beginning of the experiment, and analyzed for nutrient concentration by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). All samples were analyzed by wet chemistry procedures for concentrations of CP (method 984.13; AOAC, 2006), ADF (method 973.18 modified for use in an Ankom 200 fiber analyzer, Ankom Technology Corp., Fairport, NY; AOAC, 2006), NDF (Van Soest et al., 1991; modified for use in an Ankom 200 fiber analyzer, Ankom Technology Corp.), and FA concentrations using gas chromatography (Autosystem XL Gas Chromatograph, Perkin Elmer, Inc., Waltham, MA). Calculations for TDN used the equations proposed by Weiss et al. (1992), whereas NE_m was calculated with the equations proposed by the NRC (2000). Nutritional and FA concentrations of all feedstuffs utilized in Exp. 2 are described in Table 3.1, whereas composition and nutritional profile of dietary treatments are in Table 3.2. Corn and soybean meal collected from Exp. 2 were considered to have similar nutritional and FA profile as those used in Exp. 1, whereas the same source of CSSO and prilled saturated fat were used in both experiments.

Blood samples were collected immediately before AI (d 0), and on d 7 and 15 of the experiment from either the coccygeal vein or artery into blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing freeze-dried sodium heparin. Transrectal ultrasonography (7.5-MHz transducer; 500V, Aloka, Wallingford, CT) was performed concurrently with blood sampling on d 0, 7, and 15 to verify diameter of the largest follicle (d 0), and estimate corpus luteum (CL) volume (d 7 and 15). Corpus luteum volume was estimated using the formula for volume of a sphere; volume = $4/3\pi \times (D/2)^3$, where D is the maximum luteal diameter (Cooke et al., 2009). When the CL had a cavity, the cavity volume also was calculated as a sphere and subtracted from the CL volume.

After ultrasonography on d 15, cows diagnosed without the presence of a CL on d 0, but with a CL greater than 0.38 cm^3 in volume on d 7 and 15 (2 or 3 cows per pen; CSSO, n = 20; CON, n = 24), were assigned to conceptus collection and endometrial biopsy in the uterine horn ipsilateral to the CL, following the procedures described by Cipriano et al. (2016). Selection was performed randomly when pens had ≥ 4 cows that met the aforementioned criteria. Conceptus and endometrial samples were stored into 10-mL sterile tubes containing 2 mL of RNA stabilization solution (RNAlater, Ambion Inc., Austin, TX), maintained at 4°C for 24 h, and stored at -20°C until further processing. After conceptus collection and endometrial biopsy on d 15, all cows returned to their respective pens. On d 20, blood samples were collected from the non-flushed cows (2 or 3 cows per pen; CSSO, n = 25; CON, n = 21) into PAXgene tubes (BD Diagnostics, Sparks, MD) for whole blood RNA extraction. On d 21, treatment administration and supplementation were terminated, whereas blood samples were collected from the non-flushed cows on d 30 for pregnancy evaluation.

Laboratorial analysis. Blood samples were placed immediately on ice after collection, centrifuged (2,500 × *g* for 30 min; 4°C) for plasma harvest, and stored at -20°C on the same day of collection. Samples collected on d 0, 7, and 15 were analyzed for FA concentrations using gas chromatography (Aligent 7890, Agilent Technologies, Inc.) using the procedures described by Tripathy et al. (2010). Samples collected on d 7 and 15 from cows that did not have a CL on d 0, but with a CL greater than 0.38 cm^3 in volume concurrently with blood collection (Cipriano et al., 2016), were analyzed for P4 concentrations using a chemiluminescent enzyme immunoassay (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA). All plasma samples were analyzed for P4 within a single assay, with intra-assay CV of 2.1% and minimum detectable concentration of 0.1 ng/mL. Plasma samples collected on d 30 were analyzed for pregnancy-associated glycoproteins for evaluation of pregnancy status as reported by Pohler et al. (2016).

Total RNA was extracted only from tissue samples collected from cows that had a conceptus using the TRIzol Plus RNA Purification Kit (Invitrogen, Carlsbad, CA). Quantity and quality of isolated RNA were assessed via UV absorbance (NanoDrop Lite; Thermo Fisher Scientific, Wilmington, DE) at 260 nm and 260/280 nm ratio, respectively (Fleige and Pfaffl, 2006). Reverse transcription of extracted RNA and real-time reverse transcription-polymerase chain reaction using gene-specific primers (20 pM each; Table 3.3) were completed as described by Cipriano et al. (2016). Responses from genes of interest were quantified based on the threshold cycle (C_T), the number of polymerase chain reaction cycles required for target amplification to reach a predetermined threshold. The C_T responses from conceptus and endometrial genes of interest were normalized to the geometrical mean of C_T values of (Vandesompele et al., 2002), respectively, *glyceraldehyde-3-phosphate dehydrogenase* and *ribosomal protein L19*, and *suppressor of zeste 12 homolog* and *zinc finger protein 131*. The CV for the geometrical mean of *glyceraldehyde-3-phosphate dehydrogenase* and *ribosomal protein L19* C_T values across all conceptus samples was 4.9%. The CV for the geometrical mean of suppressor of *zeste 12 homolog* and *zinc finger protein 131* C_T values across all endometrial samples was 4.7%. Results are expressed as relative fold change ($2^{-\Delta\Delta CT}$), as described by Ocón-Grove et al. (2008).

Total RNA was extracted from whole blood samples using the PAXgene Blood RNA Kit (Qiagen, Valencia, CA). Assessment of quantity and quality of isolated RNA, reverse transcription, and real-time reverse transcription-polymerase chain reaction with gene-specific primers (20 pM each; Table 3.3) were performed as in Cipriano et al. (2016). Responses from genes of interest were quantified based on C_T and normalized to the geometrical mean of C_T *values from β2-microglobulin and β-actin* (Vandesompele et al., 2002). The CV for the geometrical mean of *β2-microglobulin and β-actin* C_T values across all blood samples was 1.8%. Results are expressed as relative fold change ($2^{-\Delta\Delta CT}$) as in Ocón-Grove et al. (2008).

Statistical Analyses Quantitative and binary data were analyzed, respectively, with the MIXED and GLIMMIX procedures of SAS (SAS Inst., Inc., Cary, NC), and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. Data from Exp. 1 were analyzed using group as experimental unit, whereas model statements contained the effect of treatment and included group(treatment × location), cow(group), and location as random variables. Pregnancy rates to timed AI also included estrus expression as an independent covariate, as well as sire and AI technician as random variables. Data from Exp. 2 used pen as experimental unit, as well as pen(treatment × group), cow(pen), and group as random variables. The model statement used for diameter of the largest follicle, estrus expression, presence of conceptus, conceptus length, and all endometrial and conceptus gene expression results contained the effect of treatment. The model statement used for blood gene expression results contained the effects of treatment, pregnancy status on d 30, and the resultant interaction. The model statement used for CL volume, plasma P4 and FA concentrations, and proportion of cows without a CL on d 0 but with CL greater than 0.38 cm^3 in volume on d 7 and 15 contained the effects of treatment, day, and the resultant interaction. Plasma FA concentrations were analyzed using values from d 0 as an independent covariate, whereas all reproductive variables included estrus expression as independent covariate. The specified term for the repeated measure analyses was day, cow(group) was the subject, and the covariance structure used was first-order autoregressive, which provided the smallest Akaike Information Criterion and hence the best fit for the variables analyzed. Results are reported as least square or covariately-adjusted least square means when appropriate, and separated using LSD. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and ≤ 0.10. Results are reported according to main treatment effect if no interaction containing the treatment effect was significant, or according to highest-order interaction detected.

3.3. Results and Discussion

3.3.1. Experiment 1

Cow age, days post-partum, and BCS on d -10 of the experiment were did not differ ($P \geq 0.59$) between CSSO-supplemented and CON cows (Table 3.4). Moreover, all cows utilized herein were in adequate nutritional status according to their BCS, and within the recommended voluntary waiting period for *B. taurus* cattle to optimize pregnancy rates to timed AI and maintain a 365-d calving interval (Short et al., 1990; Hess et al., 2005). In addition, no treatment differences were detected in estrus expression from d -3 to 0 according to activation of estrus detection patches (Thomas et al., 2014; Table 3.4), although treatment administration began after the period of estrus expression evaluation. Nevertheless, estrus expression affects pregnancy rates to timed AI in beef cows (Perry et al., 2005; Whittier et al., 2013; Thomas et al., 2014), and for this reason was included as independent covariate in the pregnancy analysis. Therefore, treatment effects reported herein for reproductive performance were not related to inherent differences in estrus expression between CSSO-supplemented and CON cows.

Cows supplemented with CSSO had greater ($P < 0.01$) pregnancy rates to timed AI compared with CON cows (Table 3.4), supporting our hypothesis and corroborating our previous research in *B. indicus* cattle (Lopes et al., 2009; Lopes et al., 2011). Moreover, the treatments utilized herein were isocaloric, isonitrogenous, and isolipidic, whereas BCS change and BCS at pregnancy diagnosis did not differ ($P \geq 0.81$; Table 3.4) between CON and CSSO-supplemented cows. Collectively, these results corroborate that CSSO supplementation enhances pregnancy success in beef cattle beyond its contribution to energy and fat intake (Lopes et al., 2009; Lopes et al., 2011).

3.3.2. Experiment 2 - Plasma FA concentrations

As in Exp. 1, cow age, days post-partum, body weight, and BCS on d -10 of the experiment (Table 3.5) did not differ between CSSO and CON cows, indicating that any treatment effects reported herein were independent of these variables. Plasma concentrations of individual and total identified FA did not differ ($P \geq 0.18$; data not shown) between CSSO-supplemented and CON cows on d 0, indicating plasma FA concentrations and profile before timed AI did not differ between treatments. During the experimental period, CSSO-supplemented cows had greater ($P < 0.01$) mean concentrations of plasma linoleic, PUFA, linoleic:linolenic ratio, and ω-6 FA compared with CON cows (Table 3.5). In turn, CON cows had greater ($P \leq 0.02$) mean concentrations of plasma palmitoleic, oleic, linolenic, docosadienoic, and ω-3 FA, and tended ($P = 0.09$) to have greater mean concentration of plasma mystiric acid compared with CSSO-supplemented cows (Table 3.5). No treatment difference was detected ($P = 0.24$) for total FA, given the isolipidic content of treatments. As in Cipriano et al. (2016), these results corroborate the FA content and profile of the CSSO treatment (Table 3.2; predominantly linoleic acid), given that plasma FA concentrations directly reflect intake and duodenal flow of FA (Lake et al., 2007; Scholljegerdes et al., 2007; Hess et al., 2008). Previous research similarly reported that CSSO supplementation increased plasma concentrations of linoleic acid, ω-6 FA, and total PUFA in beef cattle while reducing plasma concentrations of linolenic acid and ω-3 FA (Cooke et al., 2011; Cooke et al., 2014). Hence, supplementing 100 g of CSSO to *B. taurus* beef cows herein effectively increased intake and circulating concentrations of linoleic and ω-6 FA, as previously observed in *B. indicus* beef cows reared in tropical environments with diets based on warm-season feed ingredients (Cooke et al., 2014; Cipriano et al., 2016).

3.3.3. Experiment 2 - Ovarian variables and plasma P4 concentration

None of the cows evaluated herein had a CL on d 0 of the experiment. The proportion of CSSO-supplemented and CON cows that had a CL greater than 0.38 cm^3 in volume on d 7 and 15 did not differ ($P \geq 0.65$; Table 3.6), indicating that treatment effects on plasma P4 concentration and CL volume were evaluated using a balanced dataset. The same ($P = 0.99$) proportion of CSSO-supplemented and CON cows expressed estrus from d -3 to 0 (Table 3.6), whereas estrus expression did not differ (data not shown) between treatments within cows assigned ($P = 0.62$) to conceptus flushing, or within cows assigned to flushing that had a conceptus collected on d 15 ($P = 0.88$). Despite not differing between treatments and evaluated before treatment administration, estrus expression was included into all reproductive analyses as independent covariate because of its impacts on ovarian dynamics (Sá Filho et al., 2010), conceptus development, and expression of genes associated with pregnancy establishment in endometrial and conceptus tissues (Davoodi et al., 2015).

Diameter of the largest follicle on d 0 did not differ ($P = 0.51$) between CSSO-supplemented and CON cows (Table 3.5), and thus did not influence the impact of treatments on CL development and circulating P4 (Vasconcelos et al., 2001). In addition, no treatment differences were detected ($P \geq 0.73$) for plasma P4 concentration and CL volume during the experiment (Table 3.6), differing from Cooke et al. (2014) and Cipriano et al. (2016). Previous authors reported greater CL volume and plasma P4 concentrations by 15 d after timed AI in *B. indicus* cows supplemented with CSSO, and related such outcomes to improved pregnancy success reported by Lopes et al. (2009, 2011). One could attribute these discrepant results to physiological differences among *B. taurus* and *B. indicus* breeds, including hastened CL development observed in *B. taurus* cattle (Carvalho et al., 2008). Alternatively, treatments evaluated herein were isolipidic and dietary lipid content modulates CL development and steroidogenesis (Hawkins et

al., 1995). Lopes et al. (2009) also reported that *B. indicus* cows receiving 100 g/d of CSSO for 28 d after AI (d 0) had greater pregnancy rates on d 30 compared with cohorts receiving 100 g/d of Ca salts of saturated fat, whereas serum P4 concentrations on d 7 did not differ between these treatments. Collectively, these outcomes diverge from Cipriano et al. (2016) by providing evidence that CSSO-supplementation improves reproductive function and performance (as in Exp. 1) in *B. taurus* and *B. indicus* cows without increasing circulating P4 concentrations during early gestation.

3.3.4. *Experiment 2 - Pregnancy development and establishment factors*

Experiment 2 was not designed to compare pregnancy rates to timed AI between treatments based on sample size and sampling schedule, although such outcomes are reported in Table 3.6 and did not differ ($P \leq 0.26$) between CSSO-supplemented and CON cows. No treatment differences were detected ($P = 0.97$) for conceptus length (Table 3.6), which contradicts Cipriano et al. (2016) where CSSO supplementation doubled the length of conceptus on d 15 of gestation. Indeed, ω-6 FA have been show to hasten early embryonic development (Thangavelu et al., 2007) and play important roles in conceptus development by maintenance of cell metabolism, membrane fluidity, permeability, and conformation (Leroy et al., 2014; Ribeiro et al., 2016). Alternatively, average conceptus length across treatments were 11.4 ± 1.9 cm herein and 2.4 ± 0.5 cm in Cipriano et al. (2016). These suggest that on d 15 of gestation, *B. taurus* conceptus are at an advanced stage of elongation compared with *B. indicus* conceptus, and perhaps past the stage in which conceptus growth is enhanced by CSSO supplementation and ω-6 FA incorporation (Cooke et al., 2014). Supporting this rationale, Cooke et al. (2014) reported that conceptuses length and weight did not differ between CSSO-supplemented or non-supplemented *B. indicus* cows on d 19 of gestation.

A treatment effect was detected for mRNA expression of IFNt in the conceptus, which was greater ($P = 0.05$) in conceptuses from CSSO-supplemented vs. CON cows (Table 3.7). This result supports our hypothesis that CSSO supplementation enhances the IFNt-signaling cascade

(Thatcher et al., 1995) and increases pregnancy success as in Exp. 1, corroborating with similar outcomes in *B. indicus* cattle consuming tropical feed ingredients (Lopes et al., 2009; Lopes et al., 2011; Cipriano et al., 2016). These outcomes were independent of conceptus length and plasma P4 concentration, which may also increase IFNt synthesis by the conceptus (Bilby et al., 2004; Mann et al., 2006) but did not differ between treatments (Table 3.6) herein. In contrast, no treatment effects were detected (P = 0.30) for mRNA expression of *prostaglandin E synthase* (Table 3.7) in the conceptus; a rate-limiting enzyme in the synthesis of prostaglandin E_2 (Park et al., 2006). This prostaglandin is derived from ω-6 FA (Schmitz and Ecker, 2008) and produced by the conceptus and endometrium, and seems to be fundamental for conceptus development and pregnancy signaling to maternal tissues by modulating synthesis and endometrial activity of IFNt (Erdem and Guzeloglu, 2010; Dorniak et al. 2011). Cipriano et al. (2016) also reported that CSSO supplementation increased mRNA expression of *prostaglandin E synthase* in conceptuses collected on d 15 of gestation. As stated for conceptus length, perhaps conceptus collected herein (d 15; *B. taurus* conceptus) were beyond the elongation stage when CSSO supplementation modulates mRNA expression of *prostaglandin E synthase.* In endometrial samples, no treatment effects were detected for mRNA expression of *cyclooxygenase-2* and *prostaglandin E synthase* (Table 3.7), as in Cipriano et al. (2016) and Cooke et al. (2014). Hence, these results imply that CSSO supplementation to *B. taurus* beef cows increases expression of IFNt in the conceptus, without modulating expression of prostaglandin-related genes in conceptus and endometrial tissues on d 15 of gestation.

Treatment × pregnancy status interactions were detected ($P \leq 0.01$) for blood mRNA expression of the interferon-stimulated genes (ISG) *interferon-stimulated gene 15* and *20,50-oligoadenylate synthetase* on d 20 of the experiment (Table 3.7). Expression of these ISG were greater ($P \leq 0.04$) for CSSO-supplemented compared with CON cows diagnosed as pregnant, but

did not differ ($P \geq 0.27$) between treatments within cows diagnosed as non-pregnant on d 30. No treatment effects, however, were detected ($P \geq 0.48$) for blood mRNA expression of the ISG *myxovirus resistance 2*. Interferon-tau synthesis by the conceptus upregulates mRNA expression of ISGs in circulating blood leukocytes (Stevenson et al., 2007; Gifford et al., 2008; Green et al., 2010). For this reason, mRNA expression of ISGs in whole blood has been used to evaluate IFNt production and conceptus development from d 15 to 22 of gestation, as well as pregnancy diagnosis on d 18 of gestation (Fricke et al., 2016). Indeed, cows diagnosed as pregnant had greater ($P < 0.01$) expression of ISGs compared with cows diagnosed as non-pregnant within and across treatments (Table 3.7). Cipriano et al. (2016) also reported that CSSO supplementation increased mRNA expression of ISGs on d 20 of gestation in *B. indicus* beef cows, including *myxovirus resistance 2.* In this experiment, greater mRNA expression of *interferon-stimulated gene 15* and *20,50-oligoadenylate synthetase* in CSSO-supplemented cows on d 20 agree with treatment effects detected for IFNt mRNA expression in the conceptus on d 15, despite lack of similar outcomes for *myxovirus resistance 2.* These outcomes provide further support that CSSO supplementation enhances IFNt synthesis by the conceptus during the pregnancy recognition period (Spencer and Bazer, 2004; Fricke et al. 2016) in *B. taurus* beef cows.

3.4. Overall Conclusions

In summary, supplementing *B. taurus* beef cows with 100 g of CSSO for 21 d after timed AI increased pregnancy rates compared with cohorts receiving a isocaloric, isonitrogenous, and isolipidic supplements based on prilled saturated fat (Exp. 1). Moreover, CSSO supplementation increased plasma concentrations of linoleic acid and ω-6 FA, and upregulated mRNA expression of IFNt by the conceptus on d 15 of gestation (Exp. 2), which likely facilitated the increase in pregnancy rates observed in CSSO-supplemented cows from Exp. 1. Collectively, these outcomes corroborate the reproductive benefits of CSSO supplementation to *B. indicus* beef cows previously

reported by our research group (Lopes et al., 2009; Lopes et al., 2011; Cooke et al., 2014; Cipriano et al., 2016). This experiment also provided novel insights regarding potential differences in conceptus development between subspecies, which may have contributed to the lack of CSSO supplementation effects on conceptus length and mRNA expression of *prostaglandin E synthase* herein. Collectively, these research efforts validate that supplementing CSSO for 21 d beginning at timed AI is an alternative to enhance pregnancy establishment and overall reproductive performance of *B. taurus* and *B. indicus* beef cows managed, respectively, in temperate and tropical environments.

3.5. References

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4. SUPPLEMENTING CA SALTS OF SOYBEAN OIL TO LATE-GESTATING BEEF COWS: IMPACTS ON PERFORMANCE AND PHYSIOLOGICAL RESPONSES OF THE OFFSPRING*

4.1. Introduction

Maternal nutrition is a major extrinsic factor programming nutrient partitioning and development of fetal organ systems associated with health, production, and reproduction (Long et al., 2010; Silvestre et al., 2011; Garcia et al., 2014). Accordingly, nutritional management of late-gestating beef cows has been shown to directly impact performance of the subsequent offspring via programming effects (Funston et al., 2010; Bohnert et al., 2013; Marques et al., 2016). However, the majority of the research conducted within this subject focused on energy and protein nutrition, and limited information exists about the potential impacts of supplementing polyunsaturated fatty acids (PUFA) to gestating cows on offspring productivity.

Research from our group reported that supplementing Ca salts of ω-3 and ω-6 PUFA to beef cows during late-gestation improved offspring performance (Marques et al., 2017). More specifically, calves born from cows supplemented with ω-3 + ω-6 PUFA had greater average daily gain in the feedlot, and increased hot carcass weight (HCW), marbling, and longissimus muscle (LM) area compared with cohorts from non-supplemented cows. These results were suggestive of programming effects from ω-3 + ω-6 PUFA supplementation, by enhancing fetal skeletal muscle hypertrophy and adipocyte development during gestation, which translated into increased growth and marbling when offspring were provided high-energy anabolic feedlot diets (Harper and Pethick, 2004; Du et al., 2010). Nonetheless, the mechanisms underlying the outcomes reported by Marques et al. (2017) still warrant investigation, including the specific role of ω-3 and ω-6 PUFA.

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Linoleic acid and its ω-6 PUFA derivatives have been associated with cell differentiation and development in young cattle (Mangrum et al. 2016; Schubach et al., 2019). Ricks et al. (2020) recently reported that supplementing ω-6 PUFA to beef cows during the last trimester of gestation, via Ca salts of soybean oil (CSSO), increased offspring growth up to weaning. These authors, however, did not evaluate post-weaning performance of the offspring. In contrast, supplementing Ca salts of ω-3 PUFA to gestating ewes had limited benefits to lamb pre-weaning and post-weaning development (Coleman et al., 2018; Carranza-Martin et al., 2018). Therefore, supplementing CSSO to gestating beef cows may be more advantageous than the combination of ω-6 + ω-3 PUFA used by Marques et al. (2017), and will help elucidate the specific programing roles of ω-6 PUFA. Based on this rationale, we hypothesized that CSSO supplementation to late-gestating beef cows will improve life-long offspring productivity via programming effects. This experiment compared growth, physiological responses, and carcass characteristics of offspring from cows supplemented or not with CSSO during late-gestation.

4.2. Materials and Methods

This experiment was conducted at the Oregon State University – Eastern Oregon Agricultural Research Center (Burns station). The animals utilized were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University, Institutional Animal Care and Use Committee (#4974). A summary of the experimental design is presented in Figure 4.1.

4.2.1. Cow-calf management and dietary treatments

One hundred four multiparous, non-lactating, pregnant Angus × Hereford cows [unshrunk body weight (BW) = 505 ± 6 kg, age = 5.4 ± 0.3 yr, body condition score (BCS) = 4.88 ± 0.03 according to Wagner et al., 1988] were assigned to this experiment at the end of their 2nd trimester of gestation. All cows conceived to the same fixed-time artificial insemination protocol using

semen from two Angus sires, according to the breeding management and pregnancy diagnosis described by Cooke et al. (2014). Gestation length was 195 d for all cows on d 0 of the experiment.

Prior to the beginning of the experiment (d -15), cows were ranked by sire, BW, and BCS, and assigned to 1 of 2 groups (52 cows/group) in a manner that all these variables were equivalent between groups. Groups were maintained in individual meadow foxtail (Alopecurus pratensis L.) pastures from d -15 until calving. Grass-alfalfa hay was provided daily at 12.7 kg/cow (dry matter basis), and cows had ad libitum access to water and a commercial mineral + vitamin mix (Cattleman's Choice; Performix Nutrition Systems, Nampa, ID) containing 14 % Ca, 10 % P, 16 % NaCl, 1.5 % Mg, 6000 ppm Zn, 3200 ppm Cu, 65 ppm I, 900 ppm Mn, 140 ppm Se, 136 IU/g of vitamin A, 13 IU/g of vitamin D3, and 0.05 IU/g of vitamin E. No forage was available for grazing due to previous hay harvest and snow cover resultant from wintery conditions.

Cows within groups were again ranked by sire, BW, and BCS, and assigned to receive (dry matter basis) 415 g of soybean meal per cow daily in addition to 1) 195 g/cow daily of CSSO (Essentiom; Church and Dwight Co., Inc., Princeton, NJ; n = 52) or 2) 170 g/cow daily of prilled saturated fat (EnergyBooster, Milk Specialties, Eden Prairie, MN) + 25 g/cow daily of limestone (CON, n = 52). Treatments were formulated to be isocaloric, isonitrogenous and isolipidic but differing in fatty acid (FA) composition. Limestone was added to CON to compensate for the Ca included in the CSSO source. From d 0 of the experiment until calving, cows from both groups were gathered 3 times weekly (Mondays, Wednesdays, and Fridays; Cook et al., 2017; Marques et al., 2017) and individually sorted into 1 of 24 feeding pens (1 cow/pen; 6 × 9 m pens). Cows individually received treatments (1.42 kg of treatment/feeding, dry matter basis), and returned to pasture after their treatment was completely consumed. This process was repeated until all cows had been individually sorted into pens and consumed their treatments. Diets (hay + treatments)

were formulated to meet or exceed nutrient requirements for energy, crude protein, minerals, and vitamins of late-gestating beef cows (NRC, 2000; Table 4.1).

Immediately after calving, cow-calf pairs were removed from their pasture and assigned to the general management of the research herd until weaning (Marques et al., 2016), which did not include supplementation with CSSO or prilled saturated fat. Male calves were castrated at birth using an elastic castration band. All calves were administered One Shot Ultra 7 and Bovi-Shield Gold 5 (Zoetis, Florham Park, NJ) at approximately 30 d of age.

4.2.2. Calf management

Preconditioning (d 290 to 325). Calves were weaned on d 290 of the experiment and transferred to a 6-ha meadow foxtail (Alopecurus pratensis L.) pasture, which had been previously harvested for hay, for a 35-d preconditioning period as a single group. Calves were administered One Shot Ultra 7, Bovi-Shield Gold 5, and Dectomax (Zoetis, Florham Park, NJ) at weaning, and received a booster of Bovi-Shield Gold 5 and UltraChoice 7 (Zoetis) 21 d after weaning (d 311 of the experiment). During preconditioning, calves received mixed alfalfa-grass hay, water, and the same commercial mineral and vitamin mix previously described (Cattleman's Choice; Performix Nutrition Systems) for ad libitum consumption.

Growing and finishing (d 325 until slaughter). On d 325, all calves were loaded into a livestock trailer and transported for 215 km to a commercial feedyard (Cannon Hill Feeders LLC., Nyssa, OR), where they were managed as a single group until slaughter (d 514 of the experiment) at a commercial packing facility (Agri Beef Co., Toppenish, WA). Calves received a hormonal implant (Component TE 200; Elanco Animal Health, Greensfield, IN, USA) upon feedyard arrival, and were offered diets (Table 4.2) that did not contain CSSO or prilled saturated fat.

4.2.3. Sampling

Feedstuffs. Samples of all ingredients fed to late-gestating cows were collected before the beginning of the experiment and analyzed for nutrient content by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). All samples were analyzed by wet chemistry procedures for concentrations of crude protein (method 984.13; AOAC, 2006), acid detergent fiber (method 973.18 modified for use in an Ankom 200 fiber analyzer, Ankom Technology Corp., Fairport, NY: AOAC, 2006), neutral detergent fiber (Van Soest et al., 1991; modified for use in an Ankom 200 fiber analyzer, Ankom Technology Corp.), and FA concentrations using gas chromatography (Autosystem XL Gas Chromatograph, Perkin Elmer, Inc., Waltham, MA) according to Sukhija and Palmquist (1988). Only FA that were individually identified in the analysis are reported herein. Net energy for maintenance was calculated with the equations proposed by the NRC (2000).

Cows and newborn calves. Prior to the beginning of the experiment (d -15), individual unshrunk BW and BCS (Wagner et al., 1988) were recorded and a blood sample was collected from all cows via jugular venipuncture. Upon calving, cow unshrunk BW and BCS were recorded, and a blood sample collected via jugular venipuncture, while a colostrum sample was collected via hand milking (50 mL) from each cow. Concurrently with cow post-calving sampling, calf birth BW and calf gender were recorded, a blood sample was collected via jugular venipuncture, and biopsy of the LM was performed as in Schubach et al. (2019) in all calves. Cows and calves were sampled as soon as calving was completed. However, cows that calved at night and their calves were sampled at first light the next morning, but within 8 h from calving. Another blood sample was collected from calves 24 h after birth via jugular venipuncture.

Weaning and preconditioning. Cow unshrunk BW and BCS (Wagner et al., 1988) were recorded at weaning (d 290). Calf unshrunk BW was recorded over 2 consecutive days after weaning (d 290 and 291) and prior to shipping to feedyard (d 324 and 325), which were averaged

to calculate preconditioning average daily gain (ADG). Calves were observed daily for bovine respiratory disease (BRD) signs during the 35-d preconditioning period according to the subjective criteria described by Berry et al. (2004).

Feedyard. Calves were observed daily for BRD signs according to the DART system (Zoetis), and received medication according to the management criteria of the feedyard. Biopsy of the LM was again performed in all calves (Schubach et al., 2019) on d 485 of the experiment. At the commercial packing plant, HCW was collected upon slaughter. Final finishing BW was estimated based on HCW adjusted to a 63% dressing percentage (Loza et al., 2010). After a 24-h chill, trained personnel assessed carcass backfat thickness at the 12th-rib and LM area, whereas a USDA grader recorded all other carcass measures. Feedyard ADG was determined based on final preconditioning BW, and the final finishing BW estimated from HCW.

4.2.4. Laboratorial analysis

Blood samples were collected into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing either no additive or freeze-dried sodium heparin for serum and plasma collection, respectively. After collection, all blood samples were placed immediately on ice, centrifuged (2,500 × g for 30 min; 4°C) for plasma or serum harvest, and stored at -80°C on the same day of collection. Colostrum sample were also stored at -80°C on the same day of collection. All plasma samples were analyzed for FA concentration using gas chromatography (Agilent 7890, Agilent Technologies, Inc.) as in Schubach et al. (2019). Only FA that were individually identified in the analysis are reported. Colostrum and plasma samples collected from calves 24 h after birth were analyzed for IgG concentrations (E11-118; Bethyl Laboratories, Montgomery, TX).

Muscle biopsy samples were stored in 2-mL tubes containing 1 mL of RNA stabilization solution (RNAlater, Ambion, Inc., Austin, TX), and stored at -80°C until further processing. Total

RNA was extracted from muscle samples using the TRIzol Plus RNA Purification Kit (Invitrogen, Carlsbad, CA). Quantity and quality of isolated RNA were assessed via UV absorbance (NanoDrop Lite; Thermo Fisher Scientific, Wilmington, DE) at 260 nm and 260/280 nm ratio, respectively (Fleige and Pfaffl, 2006). Reverse transcription of extracted RNA and real-time reverse-transcription polymerase chain reaction (PCR) using gene specific primers (20 pM each; Table 4.3) were completed as described by Rodrigues et al. (2015). Responses from the genes of interest were quantified based on the threshold cycle (CT); the number of PCR cycles required for target amplification to reach a predetermined threshold. A portion of the amplified products were purified with the QIAquick PCR purification kit (Qiagen Inc., Valencia, CA) and sequenced at the Texas A&M AgriLife Genomics and Bioinformatics Service to verify the specificity of amplification. All amplified products represented only the genes of interest. The CT responses from muscle genes of interest were normalized to the geometrical mean of CT values of ribosomal protein S9 and β-actin (Vandesompele et al., 2002). The CV for the geometrical mean of reference genes across all samples was 2.4%. Results are expressed as relative fold change ($2^{-\Delta\Delta CT}$), as described by (Ocón-Grove et al., 2008).

4.2.5. Statistical analysis

All variables were analyzed with cow as the experimental unit, and cow(treatment × group) and group as random variables. Quantitative data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), and binary data were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc.). All data was analyzed using gestation days receiving treatment as an independent covariate, and Satterthwaite approximation to determine the denominator degrees of freedom for tests of fixed effects. Model statements for cow-related responses included the effects of treatment. Analysis of cow plasma FA profile at calving also included results from d -15 as an independent covariate. Model statements for calf-related responses analyses included

the effects of treatment, calf sex, and the treatment × calf sex interaction. Incidence of BRD signs was analyzed as repeated measures using day as fixed effect and all resultant interactions with treatment and calf sex. The subject for the repeated statement was cow(treatment × group), and the covariance structure utilized was autoregressive by providing the best fit according to the lowest Akaike information criterion. Expression of LM genes was not analyzed as repeated measures, given the substantial interval between samplings (~400 d). Results are reported as covariately-adjusted least square means, and separated using least square difference. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and ≤ 0.10.

4.3. Results and Discussion

Nutrient composition and profile of diets (hay + treatment) offered to CSSO- and CON-supplemented cows are described in Table 4.1. The CSSO was supplemented herein at the same daily amount that Marques et al. (2017) provided the mix of Ca salts of ω-3 and ω-6 PUFA to late-gestating cows. The CON treatment was included to serve as an iso-lipidic, iso-caloric, and iso-nitrogenous control. Both CSSO and CON diets were formulated to represent a typical forage-based diet with limited FA content, and provided adequate amounts of energy and crude protein to pregnant cows during last trimester of gestation (NRC, 2000). Hence, results from this experiment should not be associated with differences in total FA intake, but with the potential impacts of supplemental ω-6 PUFA to late-gestating beef cows.

4.3.1. Cow parameters

Cow age, days receiving treatments, and gestation length did not differ ($P \geq 0.60$) between CSSO and CON cows (Table 4.4). As designed, CSSO and CON cows had similar ($P \geq 0.89$) initial BW and BCS (d -15), which remained similar ($P \geq 0.40$) between treatment groups at calving and weaning. These outcomes were expected given that CSSO and CON cows consumed similar amounts of energy and protein during late-gestation, and were managed as a single group

from calving until weaning. Others have also reported similar BW and BCS responses between beef (Marques et al., 2017; Ricks et al., 2020) and dairy cows receiving supplements containing CSSO or other FA sources during late-gestation (Salehi et al., 2016; Garcia et al., 2014).

Cows assigned to receive CSSO and CON had similar ($P \geq 0.15$) plasma concentrations of individual and total FA on d -15 (data not shown); hence, equivalent circulating FA profile before treatment administration. Upon calving, CSSO cows had greater ($P < 0.01$) concentrations of plasma palmitic, stearic, linoleic, dihomo-γ-linolenic, arachidonic, and osbond acids, as well as total saturated FA, PUFA, ω-6 PUFA, and total FA compared with CON-supplemented cows (Table 4.5). Cows receiving CON had greater ($P < 0.01$) concentrations of plasma myristic, palmitoleic, oleic, and α-linolenic acids, as well as total monounsaturated and ω-3 PUFA compared with CSSO cows (Table 4.5). These results corroborate the FA content and intake of treatments, given that plasma FA profile reflects intake and intestinal flow of FA (Klusmeyer and Clark, 1991; Lake et al., 2007; Hess et al., 2008). The decrease in plasma α-linolenic acid and ω-3 PUFA concentrations in CSSO-supplemented cattle has also been reported by our group in research with mature and growing beef cattle (Brandão et al., 2018; Schubach et al., 2019; Brandão et al., 2020).

Colostrum IgG concentration was greater ($P = 0.02$) in CSSO vs. CON cows (Table 4.6). Ricks et al. (2020) also reported increased colostrum IgG concentrations in cows supplemented with CSSO during the last trimester of gestation compared with cows receiving an iso-caloric and iso-nitrogenous diet containing corn gluten feed. Supplementing CSSO and other ω-6 PUFA sources regulates immune responses in cattle, including proinflammatory reactions that stimulate humoral responses (Hess et al., 2008; Schmitz and Ecker; 2008; Garcia et al., 2016). The majority of the IgG in the colostrum of cows is derived from their systemic synthesis and circulatory reserves (Hurley and Theil, 2011). Perhaps the increased colostrum IgG concentrations in beef

cows receiving CSSO noted herein and by Ricks et al. (2020) are a resultant from heightened humoral immunity. Nonetheless, research investigating the impacts of supplementing CSSO or other ω-6 PUFA sources to late-gestating cows on colostrum quality is limited, and deserves further investigation given its importance to life-long offspring development (Besser and Gay, 1994; Wittum and Perino, 1995).

4.3.2. Calf birth, weaning, and preconditioning parameters

All cows assigned to the experiment on d -15 gave birth to a live calf; hence, calving rate was 100% across treatments (Table 4.6). No treatment effects were detected ($P \geq 0.36$) for calf birth BW and proportion of male calves born (adjusted or not; BIF, 2010), whereas calf sex influence birth BW and subsequent growth responses (Koger and Knox, 1945; Table 4.6). Calves from CSSO cows had greater ($P < 0.01$) concentrations of plasma linoleic, dihomo-γ-linolenic, and arachidonic acids, as well as total PUFA and ω-6 PUFA compared with calves from CON-supplemented cows (Table 4.7). Calves from CON cows had greater ($P \leq 0.05$) concentrations of plasma palmitoleic and α-linolenic acids, as well as total monounsaturated and ω-3 PUFA compared with CSSO cohorts (Table 4.7). These results corroborate differences noted in plasma FA profile of CSSO and CON cows at calving, given that maternal circulating FA are transferred to the fetus via the placenta (Noble et al., 1978; Garcia et al., 2014). These differences could also be associated with colostrum FA profile as a few calves may have nursed cows before sample collection, particularly those born at night. Accordingly, Ricks et al. (2020) reported greater concentrations of linoleic acid and PUFA in colostrum from CSSO-supplemented cows, and colostrum FA content reflects cow prepartum FA intake and circulating FA profile (Long et al., 2009; Leiber et al., 2011; Garcia et al., 2014). Nonetheless, Ricks et al. (2020) also collected blood samples from calves immediately after birth and before calves were capable of standing and suckling. These authors also reported greater serum concentrations of linoleic and arachidonic acid

in calves born from CSSO-supplemented cows, corroborating the differences in calf plasma FA profile noted herein (Table 4.7).

Calves from CSSO cows had greater ($P \leq 0.05$) mRNA expression in the LM of adipocyte fatty acid-binding protein (FABP4) and stearoyl-CoA desaturase (SCD) at birth, as well as tendency for the same outcome for peroxisome proliferator-activated receptor gamma (PPARγ) compared with CON cohorts (Table 4.8). No treatment effect was noted ($P = 0.36$) for mRNA expression of fatty acid synthase in the LM at birth (Table 4.8). The CT values of housekeeping genes, analyzed individually or as geometrical mean, did not differ ($P \geq 0.95$) between CSSO and CON calves at birth (22.66 vs. 22.69 for B-actin, SEM = 0.14; 19.41 vs. 19.45 for ribosomal protein S9, SEM = 0.10; 20.99 vs. 20.99 for the geometrical mean, SEM = 0.11), corroborating the validity of these mRNA expression analyses. All genes of interest analyzed in this experiment are associated with adipogenic activities in the LM (Schubach et al., 2019). More specifically, PPARγ regulates adipogenesis and lipid metabolism through induction of genes mediating these processes (Houseknecht et al., 2002), and has been identified as a candidate gene related to adipogenesis of bovine intramuscular adipose tissue (Lim et al., 2011). The FABP4 is a target gene of PPARγ (Taniguchi et al., 2008), involved in adipocyte differentiation, lipid hydrolysis, and acts as an intracellular FA chaperone (Michal et al., 2006). The SCD is a key regulatory enzyme in the lipogenic pathway modulated by PPARγ (Ntambi, 1999), and increased expression is associated with adipocyte hypertrophy (Martin et al., 1999). Linoleic acid and ω-6 PUFA regulate PPARγ mRNA expression in a ligand dependent manner (Houseknecht et al., 2002), stimulating PPARγ function and mRNA expression (Xu et al., 1999; Thoennes et al., 2000; Spurlock et al., 2000). Hence, CSSO supplementation to late-gestation cows increased supply of ω-6 PUFA to the fetus, resulting in increased expression of LM genes within the PPARγ lipogenic pathway at birth.

Calves from CSSO cows also had greater ($P \leq 0.04$) mRNA expression in the LM of myogenic differentiation 1 (MyoD) and myogenin at birth compared with CON cohorts (Table 4.8). Myogenin and MyoD are regulatory factors expressed by myocytes that regulate postnatal muscle growth, through differentiation and fusion with existing muscle fibers (Le Grand and Rudnicki, 2007; Perdiguero et al., 2009). Increased mRNA expression of these genes may indicate greater proliferation of myocytes in the LM of CSSO offspring at birth, which terminally develop into muscle fibers upon myogenin expression (Du et al., 2010; Du et al., 2011). The mechanisms by which ω-6 PUFA upregulates these genes deserve further investigation, as ω-3 PUFA are typically associated with increased expression of LM genes that regulate muscle development and function (Hiller et al., 2012). Perhaps increased supply of ω-6 PUFA during gestation to CSSO offspring promoted differentiation and development of muscle cells via proinflammatory pathways (Cooke, 2019). Arachidonic acid is a precursor of prostaglandin E2 via the cyclooxygenase-2 pathway, which promotes myogenesis in skeletal muscle by stimulating myoblast proliferation (Bondesen et al., 2004; Mo et al., 2015; Ho et al., 2017). Accordingly, calves from CSSO cows had greater concentrations of arachidonic acid compared with CON cohorts after birth, although research is warranted to validate this rationale in beef cattle.

Calves from CSSO cows had greater plasma concentrations of IgG at 24 h after birth compared with CON cohorts (Table 4.6). These outcomes are partially resultant from increased colostrum IgG concentration in CSSO cows, given that circulating IgG concentration in nursing calves are positively correlated with colostrum IgG when colostrum intake is not limited (Devery-Pocius and Larson, 1983; Morin et al., 1997). Moreover, calves from CSSO cows likely had greater ability to absorb colostrum IgG, as PUFA incorporated into intestinal cells upregulates IgG receptors (e.g. neonatal Fc receptor) responsible for IgG absorption in neonates (Israel et al., 1997; Mayer et al., 2002). Ricks et al. (2020) also reported greater serum IgG concentrations 24 h after

birth in calves born from CSSO-fed cows in their study, and stated that increased passive transfer of IgG due to CSSO supplementation could help mitigating subsequent calf morbidity and mortality.

No treatment differences were noted herein ($P \geq 0.17$) for weaning rate and proportion of male calves weaned, as well as preconditioning ADG and final BW (Table 4.7). No BRD signs were observed in calves during the preconditioning period. Alternatively, Ricks et al. (2020) reported greater pre-weaning growth in calves from cows receiving CSSO during gestation. This response, however, was mostly noted in primiparous cows and associated with improved milk production from supplementing fat to growing females (Bellows et al., 1999), whereas the current experiment used multiparous cows only. Supporting our results, others have also reported similar birth and weaning BW, as well as preconditioning performance in calves from multiparous cows supplemented or not with ω-6 PUFA during gestation (Banta et al., 2006; Banta et al., 2011 Marques et al., 2017). Collectively, calving to preconditioning results indicate that supplementing CSSO to late-gestating beef cows did not impact offspring birth BW and subsequent growth rates compared with CON-supplemented cohorts, despite differences noted in calf plasma FA profile, mRNA expression of LM genes, and IgG concentrations near birth.

4.3.3. Calf feedyard and carcass parameters

All calves were shipped to the packing plant on d 513 and slaughtered on d 514 of the experiment; hence, days on feed for both treatments was 188 d. A treatment × day interaction was detected for incidence of BRD signs in the feedyard ($P = 0.03$). A greater ($P \leq 0.05$) proportion of CON cattle were observed with BRD signs from d 333 to 337 of the experiment (d 8 to 12 after feedyard arrival; Figure 4.2), although cumulative BRD incidence did not differ ($P = 0.16$) between treatments (Table 4.9). No BRD signs were noted beyond 3 weeks of feedyard arrival until slaughter. Nonetheless, the incidence of calves diagnosed with BRD that required a second

antimicrobial treatment was less (P = 0.03) in calves from CSSO cows, resulting in reduced (P = 0.05) need of treatments compared with CON (Table 4.5). These results indicate improved immunocompetence of calves from CSSO-supplemented cows upon feedyard entry, when BRD incidence is typically elevated (Snowder et al., 2006). Such outcomes may be associated with supplemental ω-6 PUFA during gestation, as these FA play critical roles in immune system development, maturation, and function (Schmitz and Ecker, 2008; Cooke, 2019). Improved immunity of calves from CSSO cows should also be attributed to their greater plasma IgG concentrations 24 after birth, which positively impact calf immunity later in life (Wittum and Perino, 1995).

No treatment effects were detected (P ≥ 0.33) for mRNA expression of LM genes during the feedyard phase (Table 4.8). The CT values of housekeeping genes also did not differ (P ≥ 0.55) between CSSO and CON calves on d 485 (21.73 vs. 21.80 for B-actin, SEM = 0.08; 20.58 vs. 20.58 for ribosomal protein S9, SEM = 0.06; 21.15 vs. 21.19 for the geometrical mean, SEM = 0.04), further validating these mRNA expression analyses. Therefore, CSSO supplementation to late-gestating cows appears to modulate mRNA expression of adipogenic and myogenic genes in the calf LM at birth, without continuing effects later in life when supplemental CSSO or ω-6 PUFA are not provided to offspring. Treatment × sex interactions were detected (P ≤ 0.03) for feedyard ADG, final BW, and HCW (Table 4.9). These responses were greater (P ⁻≤ 0.05) in steers from CSSO cows compared with CON, and did not differ (P ≥ 0.59) between heifers (Table 4.9). Proportion of male calves slaughtered did not differ among treatments (P = 0.56), and were equal to proportion of male calves weaned (Table 4.7) given that no calf mortality was observed in the feedyard. A treatment effect was detected (P = 0.03) for carcass LM area, which was greater in calves from CSSO cows compared with CON across sexes (Table 4.9). Different than ADG and HCW, the treatment × sex interaction was not significant for this latter variable (P = 0.31). The

LM area was greater (P = 0.02) in steers and tended to be greater (P = 0.10) in heifers from CSSO cows compared with CON cohorts (82.0 vs. 78.5 cm2 in steers and 82.9 vs. 80.7 cm2 in heifers, respectively; SEM = 1.1). No treatment differences were detected (P ≥ 0.43) for the remaining carcass traits evaluated, including marbling and proportion of carcasses graded as Choice (Table 4.9). Feedyard results indicate that CSSO supplementation to late-gestating beef cows improved BW gain in the male offspring only, but increased LM development across offspring sexes. The reason for the treatment × sex interaction on ADG and HCW is unclear, as steers and heifers were equally managed as a single group in the feedyard.

Treatment differences noted in LM area were observed without an equivalent response in MyoD and myogenin mRNA expression on d 485 (te Pas et al., 1999). However, myogenic factors are downregulated as cattle mature and muscle fibers are fully developed (Picard et al., 2002; Du et al., 2010; Schubach et al., 2019). Improved feedyard ADG and carcass LM area should also be associated with enhanced immunocompetence of calves from CSSO cows, given that number of BRD treatments is negatively associated with feedlot performance and carcass merit (Blakebrough-Hall et al., 2020). Maternal dietary fat content appears to impact appetite regulation in the offspring (Gupta et al., 2008), and greater feed intake of calves from CSSO cows may have contributed to their improved feedyard growth and immunity responses (Cooke, 2017). Collectively, treatment differences noted in the feedyard support our hypothesis, at least partially, as supplemental CSSO during gestation had beneficial impacts on offspring development via programming effects. Increased marbling was also expected from CSSO supplementation, given the ω-6 PUFA effects on adipocyte development (Houseknecht et al., 2002; Mangrum et al. 2016; Schubach et al., 2019) and treatment effects on adipogenic genes at birth. The major benefits of CSSO supplementation during late gestation, however, were limited to muscle growth. Accumulation of ω-6 PUFA into fetal tissues during gestation appears to have enhanced the

development of muscle fibers, which translated into increased carcass and LM growth when offspring were provided anabolic feedlot diets (Harper and Pethick, 2004).

4.4. Overall Conclusions

Supplementing forage-fed beef cows during late gestation with CSSO did not impact cow performance, calving rate, or calf birth BW. After calving, plasma concentraitons of ω-6 PUFA were greater in CSSO cows and their offspring, as well as IgG concentrations in the colostrum and in calf plasma compared with CON cohorts. Calves from CSSO cows were also born with upregulated mRNA expression of adipogenic and myogenic genes in the LM. No treatment differences in offspring growth were observed from birth to weaning and subsequent 35-d preconditioning period. Upon feedyard arrival, offspring from CSSO cows had improved response to BRD antimicrobial treatment, whereas ADG was improved in male offspring and LM area were increased across sexes compared with CON cohorts. These results are indicative of programming effects on postnatal offspring growth and health resultant from CSSO supplementation to late-gestating cows (Funston et al., 2010; Marques et al., 2017), although CSSO impacts on muscle development vs. adipogenesis and carcass marbling warrants further investigation. Nevertheless, these outcomes indicate that supplementing CSSO to beef cows during pregnancy might be a feasible alternative to optimize offspring productivity, welfare, and carcass merit in beef systems.

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5. USING LOW-MOISTURE MOLASSES-BASED BLOCKS TO SUPPLEMENT CA SALTS OF SOYBEAN OIL TO FORAGE-FED BEEF COWS*

5.1. Introduction

Supplementing Ca salts of soybean oil (CSSO) to beef cows has been associated with productive and reproductive benefits in cow-calf systems (Cooke et al., 2019). For example, CSSO supplementation to beef females during the breeding season increased incorporation of ω-6 polyunsaturated fatty acids (FA) into maternal and embryonic tissues, enhanced mechanisms related to early maternal recognition of pregnancy, leading to increased pregnancy rates (Cooke et al., 2014; Cipriano et al., 2016; Brandão et al., 2018). Supplementing CSSO to beef cows during gestation has also stimulated programming effects on postnatal offspring growth and carcass quality, improving feedlot average daily gain and carcass marbling (Marques et al., 2017). Across these experiments, CSSO was mixed with granular feed ingredients (e.g. corn) and hand-fed to cows. Hand-fed supplementation demands intensive labor and increase production costs in pasture-based systems (Miller et al., 1991), which may discourage the use of CSSO supplementation by commercial cow-calf producers.

One strategy to alleviate labor demands is with the use of low-moisture molasses-based blocks (LMB); a self-fed form of supplementation to provide energy, protein, and custom nutrients to forage-fed cattle (Moriel et al., 2019). However, self-fed supplements such as LMB have increased intake variation compared with hand-fed granular supplements (Bowman and Sowell, 1997), which may impact duodenal absorption of CSSO and accumulation of ω-6 polyunsaturated FA in the circulation (Cooke et al., 2014). The manufacturing process of LMB includes extreme heat and changes in pH, which can also decrease ruminal stability and integrity of CSSO reaching the intestine (Sukhija and Palmquist, 1990). Hence, research is warranted to determine if inclusion of CSSO into LMB will deliver equivalent amounts of ω-6 polyunsaturated and total FA to forage-

fed beef cows compared with hand-fed granular supplements. Based on this rationale, the hypothesis of this experiment is that cows receiving CSSO via LMB will have similar plasma concentrations of ω-6 polyunsaturated FA compared with cohorts receiving CSSO daily via a hand-fed granular supplement. This experiment compared feed intake, changes in body weight (BW) and body condition score (BCS), and plasma FA profile in beef cows receiving no supplementation, or CSSO via LMB or a hand-fed granular supplement.

5.2. Materials and methods

This experiment was conducted from April to July 2019 at the Texas A&M – Beef Cattle Systems (College Station, TX). All animals were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Texas A&M - Institute of Animal Care and Use Committee (#2018-0504).

5.2.1. Animals and treatments

Thirty-six non-lactating, non-pregnant, multiparous beef cows (average ¾ *Bos taurus* and ¼ *Bos indicus*; initial BW = 445 ± 9 kg; initial BCS = 5.3 ± 0.06; age = 4.9 ± 0.3 yr) were assigned to this experiment. Cows were blocked by age (block A = 3.1 ± 0.1 yr; block B = 5.1 ± 0.2 yr; block C = 7.0 ± 0.1 yr). Within each block (n = 12 per block), cows were ranked by BW and BCS and allocated to 1 of 3 drylot pens (27 × 10 m, with 6 m of linear bunk space), in a manner that pens had similar initial average BW and BCS. Therefore, 9 pens with 4 cows each were enrolled in this experiment, whereas cow age was used as block factor as dominant older cows may limit the access of younger cows to the LMB (Bowman and Sowell, 1997; Cockwill et al., 2000).

Pens were enrolled in a replicated 3 x 2 Latin square design containing 2 periods of 42 d, and a 21-d washout interval between periods. At the beginning of each period (d 0), pens within each block were randomly assigned to receive 1 of 3 treatments: 1) self-fed LMB supplement enriched with CSSO (Essentiom; Church and Dwight Co., Inc., Princeton, NJ; n = 6), 2) Hand-fed

granular supplement enriched with CSSO (Essentiom; Church and Dwight Co., Inc.) offered daily (CONC; n = 6), or 3) no supplementation (NOSUPP; n = 6). The LMB (Midcontinent Livestock Supplements Inc., Valley Mills, TX) was designed to yield a daily intake of 0.454 kg/cow (as-fed basis), and subsequent CSSO daily intake of 100 g/cow as in Brandão et al. (2018). The CONC was designed to have the same composition of the LMB, but mixed and fed daily using individual granular ingredients. Pens were not assigned to the same treatment in both periods, whereas cows were maintained as a single group in 1-hectare paddock during the washout interval. Cows received hay (*Cynodon dactylon*), water, and a mineral-vitamin mix for ad libitum consumption during both periods and the washout interval. Composition and nutritional profile of all feed ingredients and treatments are described in Tables 5.1 and 5.2.

5.2.2. *Sampling and Laboratorial Analyses*

Samples of hay, LMB, and ingredients from the CONC treatment were collected before the beginning of the experiment and analyzed for nutrient concentration by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). All samples were analyzed by wet chemistry procedures for concentrations of crude protein (method 984.13; AOAC, 2006), acid detergent fiber (method 973.18 modified for use in an Ankom 200 fiber analyzer, Ankom Technology Corp., Fairport, NY: AOAC, 2006), neutral detergent fiber (Van Soest et al., 1991; modified for use in an Ankom 200 fiber analyzer, Ankom Technology Corp.), and FA concentrations using gas chromatography (Autosystem XL Gas Chromatograph, Perkin Elmer, Inc., Waltham, MA) according to Sukhija and Palmquist (1988). Only FA that were individually identified in the analysis are reported. Calculations for total digestible nutrients used the equations proposed by Weiss et al (1992), whereas net energy for maintenance and gain were calculated with the equations proposed by the NRC (2000).

During each experimental period (d 0 to 42), hay DM intake was recorded daily from each pen by collecting and weighing offered and non-consumed hay (0700 h). All samples were dried for 24 h at 70°C in forced-air ovens to calculate DM. Pens assigned to CONC received treatments once daily (0730 h) prior to the hay feeding, whereas CONC was consumed by cows within 30 min of feeding. One LMB (90.9 kg, as-fed basis; 58.7 cm diameter × 41.2 cm height) was placed in the back of each drylot back assigned to this treatment, in a manner that cows could access the LMB from all sides. From d 0 to 13 of each period, the LMB was not weighed to allow cows to adapt and consume blocks without interference from research personnel. The LMB was weighed every other day (0730 h) from d 14 to 42, and divided by 2 to represent daily intake. The LMB was replaced by a new one once it reached 10% of its original weight. The CONC was offered at 0.454 kg/cow daily (as-fed basis; 0.420 kg of DM/cow daily) from d 0 to 13, and adjusted (d 14 to 42) in 0.057 kg/cow (as-fed basis) increments/decrements every 2 d to match LMB intake. This adjustment rate was adopted to minimize daily variation in CONC intake, complying with intake behavior typical of hand-fed granular supplements (Bowman and Sowell, 1997).

Cow BW and BCS (Wagner et al., 1988) were recorded, and blood samples were collected on d 0, 14, 28, and 42 of each period. Blood was collected from the coccygeal vein or artery into blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing freeze-dried sodium heparin. Blood samples were placed immediately on ice after collection, centrifuged (2,500 × g for 30 min; 4 °C) for plasma harvest and stored at -80 °C on the same day of collection. Plasma samples were analyzed for FA concentration using gas chromatography (Agilent 7890, Agilent Technologies, Inc.; Santa Clara, CA) using the procedures described by Brandão et al. (2018). Only FA that were individually identified in the analysis are reported.

5.2.3. *Statistical analysis*

All data were analyzed using pen as experimental unit, Satterthwaite approximation to determine the denominator degrees of freedom for tests of fixed effects, and the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Model statements contained the effects of treatment, time variable, the treatment × time interaction, in addition to period and block as independent variables. Intake results were analyzed using pen(treatment × period) as random variable, whereas all other results used pen(treatment × period) and cow(pen) as random variables. For analyses using repeated measures, the specified term was day, whereas the subject was pen(treatment × period) for intake results and cow(pen) for all other variables. The covariance structure utilized was autoregressive, which provided the best fit for these analyses according to the lowest Akaike information criterion. All results are reported as least square means, and least square differences or PDIFF were used for simple or multiple mean separation, respectively. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and ≤ 0.10.

5.3. Results and Discussion

Supplementing LMB to cattle requires an adaptation period to ensure that animals recognize the LMB as a feed source and learn how to consume the supplement (Garossino et al., 2003; Moriel et al., 2019). For this reason, LMB intake from d 0 to 13 was not measured to prevent external interferences that affect adaptation of cows to LMB. Yet, daily LMB intake during the initial 13 d was 0.846 ± 0.107 kg/cow (DM basis), and double the designed LMB intake and concurrent CONC supplementation rate (0.420 kg/cow daily; DM basis). These outcomes may be associated with the curiosity and competition of cows to explore LMB, given that cows had no previous experience with this supplementation strategy. The LMB can also be perceived as an environmental enrichment by confined cattle, and its intake heightened in cows adapting to drylot conditions (Pelley et al., 1995). Corroborating these outcomes, Moriel et al. (2019) reported greater LMB intake during the first week of supplementation in drylotted beef heifers compared

to subsequent weeks. From d 14 to 42 of the experimental period, supplement intake was designed to be similar and indeed did not differ ($P \geq 0.21$) between LMB and CONC cows (Table 5.3). The expected variation in daily intake of LMB (Bowman and Sowell, 1997) resulted in a treatment × day interaction ($P < 0.01$) described in Figure 5.1. Intake of LMB remained greater than anticipated after d 14, suggesting that cows continued to perceive the supplement as environmental enrichment (Pelley et al., 1995). Alternatively, the LMB intake observed in this experiment may have represented the actual intake of the supplement. The LMB was designed to yield a daily intake of 0.454 kg (as-fed basis; 0.408 kg of DM/cow daily) in grazing cattle, but no grazing cows were evaluated herein to serve as reference for LMB intake.

No treatment or treatment × day interactions were noted ($P \geq 0.40$) for hay intake, BCS, and BW among treatments (Table 5.3), although CSSO and energy supplements based on molasses-based may depress forage intake and improve BW gain (Brown, 1993; Moore et al., 1999; Cooke et al., 2011). Forage intake, however, is impacted when supplemental TDN intake is > 0.70% of BW, sugarcane molasses constitutes > 15% of the dietary DM, and supplemental fat is > 2% of diet DM (Kalmbacher et al., 1995; Moore et al., 1999; Hess et al., 2008). Based on supplement DM intake from d 0 to 42 of LMB and CONC cows (0.662 and 0.530 kg/cow daily, respectively; SEM = 0.019, $P < 0.01$), supplemental TDN intake was below 0.12% of BW, and sugarcane molasses and supplemental fat represented less than 2.8% and 1.0% of dietary DM, respectively. Based on hay + supplement intake from d 0 to 42 (Table 5.3), no differences among NOSUPP, LMB, and CONC were noted ($P \geq 0.61$) for mean daily TDN intake (8.18, 8.32, and 8.19 kg/d, respectively; SEM = 0.28) and daily CP intake (2.43, 2.36, and 2.44 kg/d, respectively; SEM = 0.08). Hence, the supplementation level adopted herein was not sufficient to affect forage intake and provide supplemental energy and protein to change BW and BCS. Nonetheless, this

experiment was designed to evaluate LMB as a carrier for CSSO, and not to investigate the impacts of LMB and CONC on cattle BW and BCS gain.

Plasma concentrations of FA reflects intake and intestinal FA flow (Klusmeyer and Clark, 1991; Lake et al., 2007; Hess et al., 2008), and FA reach target tissues for accumulation via circulation (Mattos et al., 2000; Wathes et al., 2007; Cooke et al., 2014). For these reasons, the central objective of this study was to compare plasma FA profile of NOSUPP, LMB, and CONC cows throughout the experimental period (Table 5.4 to 5.6). No treatment or treatment × day interactions were detected ($P \geq 0.20$) for plasma concentrations of myristic acid, palmitoleic acid, oleic acid, arachidonic acid, docosapentaenoic acid, and total monounsaturated FA. Previous research from our group also reported that CSSO supplementation did not increase plasma concentrations of these FA in beef cows (Cooke et al., 2014; Cipriano et al., 2016; Brandão et al., 2018). Treatment × day interactions were detected for all other individual FA and total FA concentrations ($P \leq 0.01$). Plasma FA profile on d 0 did not differ ($P \geq 0.20$) between treatments (Table 5.4 to 5.6), even when periods are analyzed independently ($P \geq 0.36$; data not shown). Hence, all cows had similar circulating FA profile at the beginning of the experiment, and the washout interval eliminated carryover effects on plasma FA profile from period 1 to 2.

Plasma FA concentrations on d 14, 28, and 42 corroborate the FA content and intake of treatments during the experiment (Table 5.4 to 5.6). On d 14, plasma concentrations of palmitic acid, stearic acid, linoleic acid, osbond acid, total saturated FA, total polyunsaturated FA, total ω-6 polyunsaturated FA, and total FA were greater ($P < 0.01$) in CONC and LMB vs. NOSUPP cows, and also greater ($P \leq 0.03$) in LMB vs. CONC cows. Plasma concentrations of α-linolenic acid and total ω-3 polyunsaturated FA on d 14 were greater ($P < 0.01$) in NOSUPP vs. LMB and CONC cows, and did not differ ($P \geq 0.84$) between the latter two treatments. As previously noted, LMB intake during the initial 14 d were beyond the expected and nearly double the supplement

intake of CONC cows, explaining differences observed between these treatments in samples collected on d 14. The decrease in plasma α-linolenic acid and ω-3 polyunsaturated FA concentrations in CSSO-supplemented cattle has also been reported by our group in research with mature and growing beef cattle (Cooke et al., 2011; Brandão et al., 2018; Schubach et al., 2019). On d 28 and 42, when CONC intake was adjusted to match LMB intake (Table 5.3), plasma concentrations of palmitic acid, stearic acid, linoleic acid, γ-linolenic acid, dihomo-γ-linolenic acid, osbond acid, total saturated FA, total polyunsaturated FA, total ω-6 polyunsaturated FA, and total FA were greater in CONC and LMB vs. NOSUPP cows and did not differ ($P \geq 0.35$) between LMB and CONC cows. Plasma concentrations of α-linolenic acid and ω-3 polyunsaturated FA remained greater ($P < 0.01$) in NOSUPP vs. CONC and LMB, and similar ($P \geq 0.55$) between CONC and LMB. Therefore, cows receiving LMB or CONC had a similar plasma FA profile when receiving the same supplementation rate, and a similar increase in linoleic and its ω-6 polyunsaturated FA derivatives compared with NOSUPP cohorts.

Collectively, inclusion of CSSO into LMB resulted in similar incorporation of ω-6 polyunsaturated and total FA in the circulation compared with CONC consumed at the same rate. These results suggest that the manufacturing process of LMB did not impair the integrity and ruminal stability of CSSO, and the daily variation noted in LMB intake did not influence circulating levels of ω-6 polyunsaturated and total FA (Cook et al., 2017). Therefore, the use of self-fed LMB appears to be a valid strategy to provide CSSO to beef cattle with reduced labor needs. Research is still warranted to evaluate and refine LMB and subsequent CSSO intake by grazing cattle, and determine if providing a CSSO-enriched LMB will improve reproductive and productive responses in cow-calf systems.

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6. OVERALL SUMMARY, CONCLUSION, AND IMPLICATIONS

Collectively, results presented herein support the supplementation of ω-6 FA as a viable strategy to increase overall productivity, profitability and sustainability of cow-calf operations and ultimately improve efficiency and productivity of beef-producing systems.

More specifically, during early gestation, supplementing ω-6 FA to beef cows after AI increased the incorporation of linoleic acid and its derivates in the conceptus and enhanced pregnancy establishment signals, interacting specially with the IFNt cascade. These physiological interactions were translated into greater pregnancy rates of cows receiving CSSO compared with cohorts consuming isocaloric, isonitrogenous and isolipic FA supplement.

Comparably, during late gestation, the offspring born from cows supplemented with CSSO during the last trimester of pregnancy had greater plasma concentration of linoleic acid and its derivates at birth compared to control animals. At birth, calves born from CSSO supplemented cows also had greater mRNA expression of adipogenic and myogenic genes in the *longissumus* muscle. Phenotypically, this was translated as greater average daily gain and heavier carcasses in the male offspring and greater *longissumus* area in all offspring, whereas no differences in fat deposition variables were observed. In addition, CSSO supplemented dams also produced colostrum with greater IgG concentrations and their calves had greater IgG plasma concentrations after nursing. This possibly explains the fewer microbial treatments needed by the CSSO calves that showed signs of BRD in the feedlot, compared to their control cohorts.

Finally, the utilization of LMB as a delivery option for CSSO was demonstrated. Beef cows offered CSSO-enriched LMB had similar plasma concentration of linoleic acid and its derivatives to cohorts receiving the hand-fed, granular form of the supplement. Despite differences in intake patterns, LMB provided a similar quantity and profile of FA of the traditional supplement form. Additionally, animals in both these supplementation schemes – LMB and hand-fed – had greater

plasma concentrations of linoleic acid and its derivates compared to cows receiving no CSSO supplementation.

In summary, provision of CSSO, as a source of ω-6 FA, through LMB to beef cows for 21 days after AI and during the last trimester of gestation is a feasible strategy ton increase productive and reproductive parameters in cow-calf operations.

However, research is still warranted to investigate in further detail specific epigenetic pathways through which linoleic acid and its ω-6 derivates exert their mode of action. Another topic for future elucidation is the differences observed in growth across male and female offspring. Finally, field trials utilizing CSSO-enriched LMB in grazing systems more representative of commercial cow-calf operations, are also of interest to fully validate the practical applicability of this tool.

Table 3.1. Nutritional and fatty acid profile (dry matter basis) of feedstuffs.[1]

Item	Corn	Soybean meal	Essentiom[3]	EnergyBooster[4]	Grass-alfalfa hay
Total digestible nutrients, %	89	81	190	218	58
Net energy for maintenance, Mcal/kg	2.17	2.32	4.86	5.79	1.33
Crude protein, %	8.92	50.9	0.72	0.42	17.4
Neutral detergent fiber, %	7.51	14.8	0.91	1.75	43.2
Fatty acids,[2] %	3.74	2.71	82.5	96.1	2.09
Palmitic (16:0), %	0.49	0.44	26.5	31.1	0.44
Stearic (18:0), %	0.06	0.10	3.33	46.1	0.09
Oleic (18:1), %	1.03	0.37	22.6	6.84	0.11
Linoleic (18:2), %	2.07	1.47	25.4	0.80	0.42
Linolenic (18:3), %	0.07	0.24	2.57	0.01	0.59

[1] Corn, soybean meal, and grass-alfalfa hay samples collected from Exp. 2 only. Values obtained from a commercial laboratory wet chemistry analysis (Dairy One Forage Laboratory, Ithaca, NY). Total digestible nutrients were calculated according to the equations described by Weiss et al. (1992). Net energy for maintenance was calculated with equations described by the NRC (2000).

[3] Church and Dwight Co., Inc. (Princeton, NJ).

[4] Milk Specialties (Eden Prairie, MN).

Table 3.2. Composition and nutritional profile of treatments.[1,2]

Item	CSSO	CON
Ingredients, g/day (as-fed)		
Ground corn	100	100
Soybean meal	100	100
Essentiom	100	0
EnergyBooster	0	87
Limestone	0	13
Nutrient profile, dry matter basis		
Dry matter, %	92.5	93.1
Total digestible nutrients,[3] %	122	122
Net energy for maintenance,[4] Mcal/kg	3.16	3.23
Crude protein, %	19.9	19.6
Neutral detergent fiber, %	7.60	7.78
Ca, %	3.27	2.81
Fatty acids, %	30.9	31.7
Palmitic (16:0), %	9.58	9.87
Stearic (18:0), %	1.25	14.23
Oleic (18:1), %	8.35	2.56
Linoleic (18:2), %	10.0	1.39
Linolenic (18:3), %	0.99	0.10
Daily intake, DM basis		
Dry matter, g	278	279
Total digestible nutrients,[3] g	338	341
Net energy for maintenance,[4] Mcal	8.77	9.03
Crude protein, g	55.2	54.9
Neutral detergent fiber, g	21.1	21.7
Ca, g	9.08	7.84
Fatty acids, g	85.84	82.69
Palmitic (16:0), g	26.6	27.6
Stearic (18:0), g	3.47	39.8
Oleic (18:1), g	23.2	7.14
Linoleic (18:2), g	27.9	3.88
Linolenic (18:3), g	2.77	0.28

[1] Based on ingredients collected in Exp 2.

[2] CSSO = daily supplementation (per cow) with 100 g of a soybean meal + 100 g of ground corn + 100 g of Ca salts of soybean oil (Essentiom, Church and Dwight Co., Inc., Princeton, NJ); CON = daily supplementation (per cow) with 100 g of a soybean meal + 100 g of ground corn + 87 g of prilled saturated fat (Energy Booster 100, Milk Specialties, Eden Prairie, MN) + 13 g of limestone.

[3] Calculated according to the equations described by Weiss et al. (1992).

[4] Calculated with equations described by the NRC (2000).

Table 3.3. Primer sequences, accession number, and reference for all gene transcripts analyzed by real-time reverse transcription-polymerase chain reaction.

Target gene	Primer sequence	Accession nº	Source
Genes of interest			
20,50-oligoadenylate synthetase			
Forward	ACCCTCTCCAGGAATCCAGT	NM 001040606	Fricke et al. (2016)
Reverse	GATTCTGGTCCCAGGTCTGA		
Cyclooxygenase-2			
Forward	TCCTGAAACCCACTCCCAACA	NM_174445	Takagi et al. (2008)
Reverse	TGGGCAGTCATCAGGCACAG		
Interferon-stimulated gene 15			
Forward	GGTATGAGCTGAAGCAGTT	NM_174366	Fricke et al. (2016)
Reverse	ACCTCCCTGCTGTCAAGGT		
Interferon-tau			
Forward	GCCCTGGTGCTGGTCAGCTA	AF238612	Rizos et al. (2003)
Reverse	CATCTTAGTCAGCGAGAGTC		
Myxovirus resistance 2			
Forward	CTTCAGAGACGCCTCAGTCG	NM 173941	Fricke et al. (2016)
Reverse	TGAAGCAGCCAGGAATAGTG		
Prostaglandin E synthase			
Forward	CGCTGCTGGTCATCAAAAT	NM_174443.2	Takagi et al. (2008)
Reverse	GGAAGGGGTAGATGGTCTCC		
Reference genes			
β-actin			
Forward	CTGGACTTCGAGCAGGAGAT	AY141970	Gifford et al. (2007)
Reverse	GGATGTCGACGTCACACTTC		
β2-microglobulin			
Forward	GGGCTGCTGTCGCTGTCT	NM_173893	Silva et al. (2008)
Reverse	TCTTCTGGTGGGTGTCTTGAGT		
Glyceraldehyde-3-phosphate dehydrogenase			
Forward	ACCCAGAAGACTGTGGATGG	NM_001034034	Cerri et al. (2012)
Reverse	CAACAGACACGTTGGGAGTG		
Ribosomal protein L19			
Forward	ATTGACCGCCACATGTATCA	NM_001040516	Monteiro et al. (2014)
Reverse	GCGTGCTTCCTTGGTCTTAG		
Suppressor of zeste 12 homolog			
Forward	GAACACCTATCACACACATTCTTGT	XM_582605	Walker et al. (2009
Reverse	TAGAGGCGGTTGTGTCCACT		
Zinc finger protein 131			
Forward	AGAAAGAAGCTTTATGAATGTCAGG	NM_001101218	Walker et al. (2009)
Reverse	GTTTATCTCCAGTGTGTATCACCAG		

Table 3.4. Performance and reproductive variables in beef cows supplemented with Ca salts of soybean oil (**CSSO**; n = 11) or prilled saturated fat (**CON**; n = 11) in Exp. 1.[1,2]

Item	CSSO	CON	SEM	*P*-Value
Cow variables				
Age, yr	5.8	5.9	0.7	0.94
Days post-partum, d	66	67	3	0.86
Body condition score[3]				
d -10	5.2	5.2	0.1	0.78
d 30	5.3	5.3	0.2	0.81
Change	0.1	0.1	0.1	0.99
Reproductive variables				
Estrus detection patch, %				
Activated	43.9 (169/383)	40.9 (157/388)	3.7	0.59
Non-activated	42.9 (165/383)	45.1 (176/388)	3.3	0.63
Lost	13.2 (49/383)	14.0 (55/388)	2.0	0.71
Pregnancy rate,[4] %	60.2 (226/383)	51.7 (193/388)	4.2	0.01

[1] Cows were enrolled in an estrus-synchronization + fixed-time AI protocol (Larson et al., 2006) from d -10 to 0. Estrus detection aids (Estrotect; Rockway Inc., Spring Valley, WI) were applied on d -7 to all cows, and occurrence of estrus was recorded at timed AI (d 0).
[2] CSSO = daily supplementation (per cow) with 100 g of a soybean meal + 100 g of ground corn + 100 g of Ca salts of soybean oil (Essentiom, Church and Dwight Co., Inc., Princeton, NJ); CON = daily supplementation (per cow) with 100 g of a soybean meal + 100 g of ground corn + 87 g of prilled saturated fat (Energy Booster 100, Milk Specialties, Eden Prairie, MN) + 13 g of limestone. Treatments were offered from d 0 (timed AI) to 21 of the experiment.
[3] According to Wagner et al. (1982).
[4] Values are least square means covariately adjusted to estrus detection patch status. Values within parenthesis represent number of pregnant cows divided by number total cows within each treatment. Pregnancy was determined by the presence of a viable fetus via transrectal ultrasonography (5.0 MHz linear transducer, Ibex Pro, E.I. Medical Imaging, Loveland, CO) between d 45 and 55 of gestation.

Table 3.5. Cow variables at the beginning of the experiment (d -11), and plasma fatty acid concentrations (µg/mL of plasma) in beef cows supplemented with Ca salts of soybean oil (**CSSO**; n = 9) or prilled saturated fat (**CON**; n = 9) in Exp. 2.[1]

Item[3]	**CSSO**	**CON**	**SEM**	***P-value***
Cow variables				
Age, yr	6.74	6.76	0.39	0.96
Days post-partum, d	64.9	61.9	2.4	0.25
Body weight, kg	582	577	9	0.71
Body condition score[2]	5.12	5.13	0.08	0.91
Plasma fatty acids[3,4]				
Mystiric (14:0)	3.17	3.50	0.12	0.09
Myristoleic (14:1)	4.36	3.87	0.65	0.61
Palmitic (16:0)	92.6	92.4	3.8	0.97
Palmitoleic (16:1)	5.60	6.62	0.22	< 0.01
Stearic (18:0)	141	146	6	0.52
Oleic (18:1)	55.3	63.3	2.3	0.02
Linoleic (18:2, ω-6)	223	149	4.7	< 0.01
Linolenic (18:3, ω-3)	94.5	114	3.3	< 0.01
Dihomo-gamma-linolenic acid (20:3, ω-6)	10.1	9.49	0.37	0.29
Arachdonic (20:4, ω-6)	13.8	14.1	0.4	0.59
Docosadienoic (22:2, ω-6)	14.5	17.1	0.7	0.03
Docosapentaenoic (22:5, ω-3)	9.50	9.73	0.50	0.75
Total saturated fatty acids	249	255	10	0.69
Total monounsaturated fatty acids	64.2	69.8	3.9	0.33
Total polyunsaturated fatty acids	371	311	11	< 0.01
ω-3	105	123	3	< 0.01
ω-6	263	192	8	< 0.01
Ratio linoleic:linolenic acid	2.53	1.56	0.03	< 0.01
Total identified fatty acids	679	642	21	0.24

[1] CSSO = daily supplementation (per cow) with 100 g of a soybean meal + 100 g of ground corn + 100 g of Ca salts of soybean oil (Essentiom, Church and Dwight Co., Inc., Princeton, NJ); CON = daily supplementation (per cow) with 100 g of a soybean meal + 100 g of ground corn + 87 g of prilled saturated fat (Energy Booster 100, Milk Specialties, Eden Prairie, MN) + 13 g of limestone. Treatments were offered from d 0 (timed AI) to 21 of the experiment.

[2] According to Wagner et al. (1982).

[3] Blood samples were collected from all cows (n = 90, being 45 per treatment) on d 0 (before the first treatment application), 7, and 15. Values obtained on d 0 served as covariate; therefore, values reported are covariately-adjusted means from d 7 and 15.

[4] Saturated fatty acids = mystiric, palmitic, and stearic acids; monounsaturated fatty acids = myristoleic, palmitoleic, and oleic acids; polyunsaturated fatty acids = linoleic, linolenic, dihomo-gamma-linolenic, arachidonic, docosadienoic, and docosapentaenoic acids.

Table 3.6. Ovarian and pregnancy variables in beef cows supplemented with Ca salts of soybean oil (**CSSO**) or prilled SFA source (**CON**) in Exp. 2.[1, 2]

Item	CSSO	CON	SEM	*P*-Value
Ovarian variables				
Largest follicle diameter (d 0), mm	16.6	15.7	0.44	0.14
Corpus luteum on d 7 and 15, %	86.1 (39/45)	92.7 (42/45)	7.4	0.29
Corpus luteum volume,[3] cm^3	7.11	7.00	0.35	0.83
Plasma progesterone,[3] ng/mL	4.20	4.35	0.32	0.73
Reproductive variables				
Estrus expression, %	38.0 (18/45)	38.0 (18/45)	21.2	0.97
Proportion of cows with conceptus,[4] %				
d 15	57.5 (11/20)	39.0 (10/24)	11.2	0.26
d 30	52.6 (12/25)	47.4 (11/21)	11.6	0.73
Conceptus length,[5] cm	11.3	11.4	3.1	0.97

[1] CSSO = daily supplementation (per cow) with 100 g of a soybean meal + 100 g of ground corn + 100 g of Ca salts of soybean oil (Essentiom, Church and Dwight Co., Inc., Princeton, NJ); CON = daily supplementation (per cow) with 100 g of a soybean meal + 100 g of ground corn + 87 g of prilled saturated fat (Energy Booster 100, Milk Specialties, Eden Prairie, MN) + 13 g of limestone. Treatments were offered from d 0 (timed AI) to 21 of the experiment. All results are covariately-adjusted to estrus expression (Estrotect; Rockway Inc., Spring Valley, WI; Thomas et al., 2014) from d -3 to 0 of the experiment. Values within parenthesis represent number of cows with a positive response divided by number total cows within each treatment.

[2] Transrectal ultrasonography (7.5-MHz transducer; 500V, Aloka, Wallingford, CT) was performed on d 0, 7 and 15 of the experiment. Blood samples were collected for progesterone analysis on d 0, 7 and 15.

[3] Evaluated in cows without a corpus luteum on d 0, but with a corpus luteum greater than 0.38 cm^3 in volume on d 7 (n = 39 for CSSO and 42 for CON) and 15. Corpus luteum volume was calculated using the formula for volume of a sphere; $V = 4/3\pi \times (D/2)^3$, where D is the maximum luteal diameter (Cooke et al., 2009).

[4] On d 15, 44 cows (CSS0, n = 20; CON, n = 24) were assigned to transcervical flushing for conceptus collection (Cipriano et al., 2016). On d 30, pregnancy status of the non-flushed cows was evaluated by measuring pregnancy associated glycoproteins in plasma (Pohler et al., 2016).

[5] Evaluated from cows that had a conceptus collected via transcervical flushing.

Table 3.7. Expression of genes associated with pregnancy establishment in the endometrium, conceptus, and blood from beef cows supplemented with Ca salts of soybean oil (**CSSO**) or prilled SFA source (**CON**) in Exp. 2.[1, 2]

Item	CSSO	CON	SEM	*P*-Value
Endometrium[2]				
Cyclooxygenase-2	4.88	5.11	1.32	0.89
Prostaglandin E synthase	5.76	7.40	1.10	0.30
Conceptus[2]				
Interferon-tau	21.3	12.1	3.4	0.05
Prostaglandin E synthase	2.22	2.50	0.48	0.69
Blood cells[3]				
Interferon-stimulated gene 15				
Pregnant	43.1	29.8	4.6	0.04
Non-pregnant	1.87	3.57	5.48	0.81
Myxovirus resistance 2				
Pregnant	20.2	20.1	2.7	0.98
Non-pregnant	2.30	4.80	3.01	0.48
20,50-oligoadenylate synthetase				
Pregnant	26.8	18.3	2.7	0.03
Non-pregnant	1.67	2.64	3.33	0.83

[1] CSSO = daily supplementation (per cow) with 100 g of a soybean meal + 100 g of ground corn + 100 g of Ca salts of soybean oil (Essentiom, Church and Dwight Co., Inc., Princeton, NJ); CON = daily supplementation (per cow) with 100 g of a soybean meal + 100 g of ground corn + 87 g of prilled saturated fat (Energy Booster 100, Milk Specialties, Eden Prairie, MN) + 13 g of limestone. Treatments were offered from d 0 (timed AI) to 21 of the experiment. All results are covariately-adjusted to estrus expression (Estrotect; Rockway Inc., Spring Valley, WI; Thomas et al., 2014) from d -3 to 0 of the experiment.

[2] Conceptus were collected via transcervical flushing and endometrial biopsy was performed on d 15 from 44 cows (CSSO, n = 20; CON, n = 24). Only samples from cows with a retrieved conceptus were analyzed. Values are expressed as relative fold change compared to threshold cycle of reference genes analyzed within the same sample (Ocón-Grove et al., 2008).

[3] Blood samples collected from non-flushed cows (CSSO, n = 25; CON, n = 21) into PAXgene tubes (BD Diagnostics, Sparks, MD) for whole blood RNA extraction on d 20 of the experiment, and analyzed according to cow pregnancy status on d 30. Values are expressed as relative fold change compared to threshold cycle of reference genes analyzed within the same sample (Ocón-Grove et al., 2008).

Table 4.1. Composition and nutritional profile of diets containing Ca salts of soybean oil (**CSSO**) or prilled saturated fat (**CON**).

Item	**CON**	**CSSO**
Ingredients, kg/d (dry matter basis)		
Grass-alfalfa hay	12.7	12.7
Soybean meal	0.415	0.415
Essentiom[1]	0	0.195
EnergyBooster[2]	0.170	0
Limestone	0.025	0
Nutrient profile,[3] dry matter basis		
Dry matter, %	91.9	92.1
Net energy for maintenance,[3] Mcal/kg	1.28	1.28
Crude protein, %	8.3	8.3
Fatty acids, %	2.46	2.45
Palmitic (16:0), %	0.64	0.63
Stearic (18:0), %	0.61	0.08
Oleic (18:1), %	0.16	0.41
Linoleic (18:2), %	0.29	0.65
Linolenic (18:3), %	0.31	0.35

[1] Essentiom (Church and DwightCo., Inc., Princeton, NJ).

[2] Energy Booster 100 (Milk Specialties, Eden Prairie, MN).

[3] Values obtained via wet chemistry analysis (Dairy One Forage Laboratory, Ithaca, NY).

Table 4.2. Ingredient composition (as-fed basis) of feedyard diets offered to cattle.

	Diets[1]				
Ingredients, % as fed	**A**	**B**	**C**	**D**	**E**
Alfalfa hay	53.0	39.0	19.0	12.0	4.0
Distillers grains	0.0	0.0	2.0	2.0	4.0
Dried corn	0.0	0.0	11.0	10.0	18.0
High-moisture corn	42.0	55.0	46.0	53.3	54.5
Corn Silage	0.0	0.0	18.0	17.0	11.0
Mineral and vitamin mix[3]	5.0	6.0	4.0	4.5	6.5
Tallow	0.0	0.0	0.0	1.2	2.0

[1] A = offered for 5 d after arrival receiving; B = offered for 5 d after diet A; C = offered for 7 d after diet B; D = offered for 13 d after diet C; E = offered for 188 d until slaughter.
[3] Customized blend of minerals, vitamins and feed additives (Performix Nutrition Systems, Nampa, ID, USA).

Table 4.3. Primer sequences, accession number, and reference for all gene transcripts analyzed by real-time reverse transcription polymerase chain reaction.

Target[1]	Primer Sequence	Accession nº
FABP4 (Li et al., 2018)		
Forward	AAACTTAGATGAAGGTGCTCTGG	AJ4160220
Reverse	CATAAACTCTGGTGGCAGTGA	
FASN (Jeong et al, 2012)		
Forward	ATCGAGTGCATCAGGCAAGT	AF479289
Reverse	TGTGAGCACATCTCGAAAGCCA	
MyoD (Muroya et al., 2002)		
Forward	ATCCTGCGCAACGCCATCCGCTATATCGA	AF093675
Reverse	CTCGCTGTAGTAAGTGCGGTCGTAGCAGT	
Myogenin (Muroya et al., 2002)		
Forward	GAGAAGCGCAGACTCAAGAAGGTGAATGA	AF091714
Reverse	TCTGTAGGGTCCGCTGGGAGCAGATGATC	
PPARγ (Li et al., 2018)		
Forward	GCATTTCCACTCCGCACTAT	AY137204
Reverse	GGGATACAGGCTCCACTTTG	
SCD (Li et al., 2018)		
Forward	GCCAACAACTCTGCCTTTATG	GU947654
Reverse	CACCAATGACTGACCACCTG	
B-actin (Bong et al, 2012)		
Forward	AGCAAGCAGGAGTACGATGAGT	NM_173979
Reverse	ATCCAACCGACTGCTGTCA	
Ribosomal protein S9 (Jeong et al., 2012)		
Forward	CCTCGACCAAGAGCTGAAG	AF479289
Reverse	CCTCCAGACCTCACGTTTGTTC	

[1] FABP4, *adipocyte fatty acid binding protein*; FASN, *fatty acid synthase*; MyoD, *myogenic differentiation 1*; PPARγ, *peroxisome proliferator activated receptor gamma*; SCD, *stearoyl-CoA desaturase*.

Table 4.4. Performance of beef cows receiving diets supplemented with Ca salts of soybean oil (**CSSO**; n = 52) or prilled saturated fat (**CON**; n = 52) during the last trimester of gestation.[1,2]

Item	**CON**	**CSSO**	**SEM**	***P* =**
Cow age, yr	5.33	5.39	0.41	0.93
Days receiving treatments, d	85.5	85.1	0.6	0.60
Gestation length, d	280	280	0.6	0.60
Body weight, kg				
Initial (d -15)	504	505	9	0.89
Calving	545	554	9	0.48
Weaning (d 290)	568	564	9	0.78
Body condition score				
Initial (d -15)	4.88	4.88	0.04	0.92
Calving	4.74	4.82	0.07	0.59
Weaning (d 290)	5.19	5.09	0.08	0.40

[1] Cows received (dry matter basis) 195 g/cow daily of CSSO (Essentiom; Church and Dwight Co., Inc., Princeton, NJ) or 170 g/cow daily of prilled saturated fat (Energy Booster 100; Milk Specialties, Eden Prairie, MN). Treatments were provided from d 0 (d 195 of gestation) to calving.

[2] Unshrunk body weight and body condition score (Wagner et. al., 1988) were recorded prior to the beginning of the experiment (initial; d -15), within 8 h of after calving, and at weaning (d 290)

Table 4.5. Plasma fatty acid (**FA**) profile (µg/mL of plasma) at calving of beef cows receiving diets supplemented with Ca salts of soybean oil (**CSSO**; n = 52) or prilled saturated fat (**CON**; n = 52) during the last trimester of gestation. [1,2]

Item	CON	CSSO	SEM	*P-value*
Myristic (14:0)	6.34	4.76	0.17	< 0.01
Palmitic (16:0)	83.0	102	3.5	< 0.01
Palmitoleic (16:1, ω-7)	10.3	4.39	0.36	< 0.01
Stearic (18:0)	98.7	113	4.0	0.01
Oleic (18:1, ω-9)	90.0	60.5	3.3	< 0.01
Linoleic (18:2, ω-6)	145	342	12	< 0.01
α-Linolenic (18:3, ω-3)	60.5	34.2	1.5	< 0.01
Dihomo-γ-linolenic acid (20:3, ω-6)	8.63	12.1	0.4	< 0.01
Arachidonic (20:4, ω-6)	13.3	19.2	0.7	< 0.01
Osbond (22:5, ω-6)	20.3	26.9	1.1	< 0.01
Docosapentaenoic (22:5, ω-3)	6.77	7.28	0.27	0.20
Total saturated FA	201	233	7	< 0.01
Total monounsaturated FA	104	67	4	< 0.01
Total polyunsaturated FA	270	450	15	< 0.01
Total ω-3 polyunsaturated FA	67.4	41.7	1.7	< 0.01
Total ω-6 polyunsaturated FA	202	408	13	< 0.01
Total identified FA	575	750	25	< 0.01

[1] Cows received (dry matter basis) 195 g/cow daily of CSSO (Essentiom; Church and Dwight Co., Inc., Princeton, NJ) or 170 g/cow daily of prilled saturated fat (Energy Booster 100; Milk Specialties, Eden Prairie, MN). Treatments were provided from d 0 (d 195 of gestation) to calving.

[2] Blood samples were collected from all cows on d -15 of the experiment and within 8 h after calving, and analyzed for fatty acid profile according as in Schubach et al. (2019). Values form d -15 were used as independent covariate within each individual FA analysis.

Table 4.6. Calving, weaning, and preconditioning responses from offspring of beef cows receiving diets containing Ca salts of soybean oil (**CSSO**; n = 52) or prilled saturated fat (**CON**; n = 52) during the last trimester of gestation.[1]

Item	**CON**	**CSSO**	**SEM**	***P-value***
Calving results				
Calving rate, %	100	100	-	-
% of male calves born	56.6	51.0	7.0	0.57
Calf birth weight, kg	37.0	37.7	0.6	0.42
Adjusted calf birth weight,[2] kg	38.3	39.1	0.6	0.36
Colostrum IgG, mg/mL	373	423	15	0.02
Calf plasma IgG 24 h after birth, mg/mL	55.7	97.9	9.0	< 0.01
Weaning results				
Weaning rate, %	96.0	100	2.0	0.17
% of male calves weaned	56.9	51.0	7.0	0.56
Calf weaning age, d	209	209	0.1	0.91
Calf weaning weight, kg	262	264	4	0.72
Calf 205-d adjusted weaning weight,[2] kg	267	270	4	0.57
Preconditioning results				
Average daily gain, kg/d	0.68	0.67	0.05	0.84
Final body weight, kg	287	289	4	0.77

[1] Cows received (dry matter basis) 195 g/cow daily of CSSO (Essentiom; Church and Dwight Co., Inc., Princeton, NJ) or 170 g/cow daily of prilled saturated fat (Energy Booster 100; Milk Specialties, Eden Prairie, MN). Treatments were provided from d 0 (d 195 of gestation) to calving. Calves were weaned on d 290 of the experiment, preconditioned for 35 d (d 291 to 325), and transferred to a feedyard (Cannon Hill Feeders LLC., Nyssa, OR) where they remained until slaughter (d 514). All body weights collected were unshrunk.

[2] According to BIF (2010).

Table 4.7. Plasma fatty acid (**FA**) profile (μg/mL of plasma) at birth from offspring of beef cows receiving diets supplemented with Ca salts of soybean oil (**CSSO**; n = 52) or prilled saturated fat (**CON**; n = 52) during the last trimester of gestation. [1,2]

Item	CON	PUFA	SEM	*P-value*
Myristic (14:0)	12.9	12.7	1.5	0.92
Palmitic (16:0)	96.5	96.9	6	0.96
Palmitoleic (16:1, ω-7)	15.9	3.6	0.8	0.04
Stearic (18:0)	36.5	36.6	1.6	0.98
Oleic (18:1, ω-9)	91.4	83.6	3.7	0.14
Linoleic (18:2, ω-6)	24.5	41.9	4.2	< 0.01
α-Linolenic (18:3, ω-3)	1.23	0.100	0.285	< 0.01
Dihomo-γ-linolenic acid (20:3, ω-6)	3.63	5.69	0.39	< 0.01
Arachidonic (20:4, ω-6)	7.98	11.6	0.69	< 0.01
Osbond (22:5, ω-6)	0.912	0.379	0.328	0.25
Docosapentaenoic (22:5, ω-3)	0.621	0.233	0.279	0.32
Total saturated FA	150	150	9	0.99
Total monounsaturated FA	121	107	5	0.05
Total polyunsaturated FA	40.1	60.6	4.6	< 0.01
Total ω-3 polyunsaturated FA	2.50	1.05	0.50	0.05
Total ω-6 polyunsaturated FA	37.6	59.5	4.4	< 0.01
Total identified FA	311	319	15	0.73

[1] Cows received (dry matter basis) 195 g/cow daily of CSSO (Essentiom; Church and Dwight Co., Inc., Princeton, NJ) or 170 g/cow daily of prilled saturated fat (Energy Booster 100; Milk Specialties, Eden Prairie, MN). Treatments were provided from d 0 (d 195 of gestation) to calving.

[2] Blood samples were collected from all cows within 8 h after calving, and analyzed for fatty acid profile as in Schubach et al. (2019).

Table 4.8. Expression of *longissimus* muscle genes in the offspring of beef cows receiving diets containing Ca salts of soybean oil (**CSSO**; n = 52) or prilled saturated fat (**CON**; n = 52) during the last trimester of gestation.[1]

Item	CON	CSSO	SEM	*P-value*
FABP4				
Birth	35.0	73.9	14	0.03
Feedyard	6.09	6.97	0.80	0.44
FASN				
Birth	1.77	1.92	0.11	0.36
Feedyard	4.36	4.61	0.44	0.69
MyoD				
Birth	13.3	22.6	3.0	0.02
Feedyard	3.76	3.89	0.30	0.76
Myogenin				
Birth	7.03	9.77	1.00	0.04
Feedyard	2.45	2.60	0.18	0.55
PPARγ				
Birth	3.16	4.55	0.56	0.07
Feedyard	2.66	3.00	0.28	0.38
SCD				
Birth	3.43	4.54	0.34	0.05
Feedyard	3.16	3.60	0.31	0.33

[1] Cows received (dry matter basis) 195 g/cow daily of CSSO (Essentiom; Church and Dwight Co., Inc., Princeton, NJ) or 170 g/cow daily of prilled saturated fat (Energy Booster 100; Milk Specialties, Eden Prairie, MN). Treatments were provided from d 0 (d 195 of gestation) to calving. Calves were weaned on d 290 of the experiment, preconditioned for 35 d (d 291 to 325), and transferred to a feedyard (Cannon Hill Feeders LLC., Nyssa, OR) where they remained until slaughter (d 514).

[2] Samples of the *longissimus* muscle were taken via needle biopsy within 8 h after birth, and on d 485 of the experiment. Values are expressed as relative fold change compared with threshold cycle of reference genes analyzed within the same sample (Ocón-Grove et al., 2008)

[3]FABP4, *adipocyte fatty acid binding protein*; FASN, *fatty acid synthase*; MyoD, *myogenic differentiation 1*; PPARγ, *peroxisome proliferator activated receptor gamma*; SCD, *stearoyl-CoA desaturase*

Table 4.9. Feedyard performance and carcass characteristics from offspring of beef cows receiving diets containing Ca salts of soybean oil (**CSSO**; n = 52) or prilled saturated fat (**CON**; n = 52) during the last trimester of gestation. [1]

Item	CON	CSSO	SEM	*P-value*
Cattle treated for respiratory disease,[2] %				
Once	40.5	28.4	6.7	0.16
Twice	19.2	5.64	4.79	0.03
Number of antimicrobial treatments required	1.49	1.18	0.10	0.05
Average daily gain, kg/d				
Steers	1.39	1.52	0.06	0.05
Heifers	1.54	1.50	0.06	0.59
Final body weight, kg				
Steers	553	579	9	0.02
Heifers	575	570	9	0.66
Carcass characteristics[3]				
Hot carcass weight, kg				
Steers	349	365	6	0.02
Heifers	362	359	6	0.66
Backfat, cm	2.46	2.40	0.14	0.76
Longissimus muscle area, cm^2	79.6	82.4	1.1	0.03
Marbling score	526	510	15	0.47
Yield grade	3.76	3.68	0.07	0.43
Carcasses grading Choice or above, %	85.9	88.0	4.9	0.77

[1] Cows received (dry matter basis) 195 g/cow daily of CSSO (Essentiom; Church and Dwight Co., Inc., Princeton, NJ) or 170 g/cow daily of prilled saturated fat (Energy Booster 100; Milk Specialties, Eden Prairie, MN). Treatments were provided from d 0 (d 195 of gestation) to calving. Calves were weaned on d 290 of the experiment, preconditioned for 35 d (d 291 to 325), and transferred to a feedyard (Cannon Hill Feeders LLC., Nyssa, OR) where they remained until slaughter (d 514).

[2] Calves were observed daily for respiratory disease signs based on the DART system (Zoetis, Florham Park, NJ), and received medication according to the management criteria of the commercial feedyard.

[3] Backfat thickness measured at the 12^{th} rib; marbling score: 400 = $Small^{00}$, 500 = $Modest^{00}$; 600 = $Medium^{00}$; yield grade calculated as reported by Lawrence et al. (2010)

Table 5.1. Nutritional and fatty acid profile (dry matter basis) of feedstuffs. [1]

Item	Cottonseed meal	Essentiom[2]	Dry molasses	Hay
Dry matter, %	89.9	95.0	91.6	74.5
Total digestible nutrients, %	68	190	77	59
Net energy for maintenance, Mcal/kg	1.58	4.86	1.87	1.23
Crude protein, %	45.3	0.70	9.50	17.5
Neutral detergent fiber, %	25.2	1.10	1.22	49.9
Fatty acids,[3] %	5.00	82.0	0.62	2.22
Palmitic (16:0), %	1.28	25.7	0.12	0.46
Stearic (18:0), %	0.16	3.08	0.04	0.09
Oleic (18:1, ω-9), %	1.05	22.9	0.12	0.30
Linoleic (18:2, ω-6), %	2.25	27.1	0.23	0.60
α-Linolenic (18:3, ω-3), %	0.04	2.51	0.08	0.40

[1] Values obtained from a commercial laboratory wet chemistry analysis (Dairy One Forage Laboratory, Ithaca, NY). Total digestible nutrients were calculated according to the equations described by Weiss et al. (1992). Net energy for maintenance was calculated with equations described by the NRC (2000).
[2] Church and Dwight Co., Inc. (Princeton, NJ).
[3] According to Sukhija and Palmquist (1988) using gas chromatography (Autosystem XL Gas Chromatograph, Perkin Elmer, Inc., Waltham, MA).

Table 5.2. Composition and nutritional profile of treatments.

Item	CONC	LMB
Ingredients, % dry matter basis		
Cottonseed Meal	8.50	8.37
Molasses	60.3	60.4
Essentiom	24.7	24.7
Ca phosphate	3.35	3.36
Mg oxide	3.15	3.17
Nutrient profile, dry matter basis		
Dry matter, %	92.7	89.9
Total digestible nutrients,[2] %	99	87
Net energy for maintenance,[3] Mcal/kg	2.46	2.20
Crude protein, %	9.75	9.30
Neutral detergent fiber, %	3.14	3.40
Fatty acids, %	21.0	21.9
Palmitic (16:0), %	6.53	6.57
Stearic (18:0), %	0.80	0.91
Oleic (18:1, ω-9), %	5.81	5.59
Linoleic (18:2, ω-6), %	7.02	7.17
α-Linolenic (18:3, ω-3), %	0.67	0.80

[1] CONC = Hand-fed granular supplement enriched with Ca salts of soybean oil (Essentiom, Church and Dwight Co., Inc., Princeton, NJ); LMB = low-moisture molasses-based block enriched with Ca salts of soybean oil (Essentiom, Church and Dwight Co., Inc.). Results are based on individual ingredients of the CONC, and LMB sample collected prior to the beginning of the experiment.
[2] Calculated according to the equations described by Weiss et al. (1992).
[3] Calculated with equations described by the NRC (2000).

Table 5.3. Feed intake, body weight, and body condition score of forage-fed beef cows receiving no supplementation (**NOSUPP**; n = 6 pens), or receiving a molasses-based supplement enriched with Ca salts of soybean oil (24.7% of dry matter; Essentiom, Church and Dwight Co., Inc., Princeton, NJ) via self-fed low-moisture block (**LMB**; n = 6 pens) or hand-fed granular concentrate daily (**CONC**; n = 6 pens). Supplement treatments were provided from d 0 to 42 of the experiment.[1]

Item	**NOSUPP**	**CONC**	**LMB**	**SEM**	***P-value***
Supplement intake, kg/d (DM basis)					
d 14 to 28	-	0.554	0.564	0.017	0.68
d 28 to 42	-	0.611	0.575	0.018	0.21
Overall (d 14 to 42)	-	0.583	0.570	0.011	0.39
Hay intake, kg/d (DM basis)	13.8	13.6	13.2	0.5	0.59
Body condition score[2]					
d 0	5.54	5.58	5.54	0.11	0.95
d 14	5.71	5.79	5.79	0.11	0.83
d 28	5.89	6.00	5.85	0.11	0.64
d 42	6.17	6.33	6.14	0.11	0.44
Total gain (d 0 to 42)	0.62	0.75	0.60	0.08	0.40
Body weight, kg					
d 0	464	463	465	20	0.99
d 14	478	476	477	20	0.99
d 28	489	486	496	20	0.93
d 42	494	482	501	20	0.81
Total gain (d 0 to 42)	29	19	35	11	0.60

[1] Hay intake was recorded daily from each pen by collecting and weighing offered and non-consumed hay. From d 0 to 13, the LMB was not weighed to allow cows to adapt and consume blocks without interference from research personnel. The LMB was weighed every other day from d 14 to 42, divided by 2 to represent daily intake and averaged across LMB pens. The CONC was offered at 0.454 kg/cow daily (as-fed basis; 0.420 kg of DM/cow daily) from d 0 to 13, and adjusted (d 14 to 42) every 2 d to match LMB intake.
[2] According to Wagner et al. (1988).

Table 5.4. Plasma concentrations of saturated and monounsaturated fatty acids (μg/mL of plasma) in forage-fed beef cows receiving no supplementation (**NOSUPP**; n = 6 pens), or receiving a molasses-based supplement enriched with Ca salts of soybean oil (24.7% of dry matter; Essentiom, Church and Dwight Co., Inc., Princeton, NJ) via self-fed low-moisture block (**LMB**; n = 6 pens) or hand-fed granular concentrate daily (**CONC**; n = 6 pens). Supplement treatments were provided from d 0 to 42 of the experiment.[1]

Item[3]	**NOSUPP**	**CONC**	**LMB**	**SEM**	***P-value***
Myristic (14:0)	5.29	5.71	4.76	0.45	0.36
Palmitic (16:0)					
d 0	73.3	69.6	74.2	5.3	0.81
d 14	80.2^{c}	101^{b}	119^{a}	5.3	< 0.01
d 28	70.4^{b}	102^{a}	98.4^{a}	5.3	< 0.01
d 42	66.1^{b}	102^{a}	97.4^{a}	5.3	< 0.01
Palmitoleic (16:1, ω-7)	3.36	3.15	3.08	0.12	0.26
Stearic (18:0)					
d 0	116	117	114	6	0.91
d 14	117^{c}	138^{b}	163^{a}	6	< 0.01
d 28	114^{b}	152^{a}	146^{a}	6	< 0.01
d 42	100^{b}	137^{a}	148^{a}	6	< 0.01
Oleic (18:1, ω-9)	45.2	49.7	50.3	2.1	0.20

[1] Blood samples were collected on d 0, 14, 28, and 42 for plasma harvest, and analyzed for fatty acid concentration according to Brandão et al. (2018)

Table 5.5. Plasma concentrations of polyunsaturated fatty acids (μg/mL of plasma) in forage-fed beef cows receiving no supplementation (**NOSUPP**; n = 6 pens), or receiving a molasses-based supplement enriched with Ca salts of soybean oil (24.7% of dry matter; Essentiom, Church and Dwight Co., Inc., Princeton, NJ) via self-fed low-moisture block (**LMB**; n = 6 pens) or hand-fed granular concentrate daily (**CONC**; n = 6 pens). Supplement treatments were provided from d 0 to 42 of the experiment.[1]

Item[3]	**NOSUPP**	**CONC**	**TUB**	**SEM**	***P-value***
Linoleic (18:2, ω-6)					
d 0	135	133	128	11	0.88
d 14	139[c]	245[b]	332[a]	11	< 0.01
d 28	141[b]	306[a]	305[a]	11	< 0.01
d 42	139[b]	313[a]	330[a]	11	< 0.01
γ-Linolenic (18:3, ω-6)					
d 0	5.25	5.02	4.76	0.33	0.58
d 14	4.41	4.43	3.72	0.33	0.24
d 28	4.63[b]	6.00[a]	6.09[a]	0.33	< 0.01
d 42	4.24[b]	5.64[a]	6.17[a]	0.33	< 0.01
α-Linolenic (18:3, ω-3)					
d 0	67.2	65.9	66.7	3.0	0.95
d 14	65.4[a]	44.6[b]	44.4[b]	3.0	< 0.01
d 28	70.0[a]	51.7[b]	51.7[b]	3.0	< 0.01
d 42	64.3[a]	46.2[b]	51.7[b]	3.0	< 0.01
Dihomo-γ-linolenic acid (20:3, ω-6)					
d 0	11.3	10.8	10.2	0.8	0.64
d 14	12.5	13.6	12.4	0.8	0.56
d 28	11.8[b]	16.3[a]	15.4[a]	0.8	0.02
d 42	10.5[c]	14.4[b]	17.0[a]	0.8	< 0.01
Arachdonic (20:4, ω-6)	19.5	19.8	19.8	0.5	0.93
Docosadienoic (22:2, ω-6)					
d 0	9.47	9.13	10.0	0.57	0.51
d 14	10.9[a]	7.97[b]	7.74[b]	0.57	< 0.01
d 28	9.78[a]	7.16[b]	7.03[b]	0.57	< 0.01
d 42	9.67[a]	6.77[b]	6.90[b]	0.57	< 0.01
Docosapentaenoic (22:5, ω-3)	9.88	9.41	9.70	0.41	0.71
Osbond (22:5, ω-6)					
d 0	17.2	16.3	16.1	1.1	0.74
d 14	17.0[c]	21.1[b]	26.3[a]	1.1	< 0.01
d 28	19.1[b]	26.2[a]	25.9[a]	1.1	< 0.01
d 42	18.7[b]	26.4[b]	29.7[a]	1.1	< 0.01

[1] Blood samples were collected on d 0, 14, 28, and 42 for plasma harvest, and analyzed for fatty acid concentration according to Brandão et al. (2018)

Table 5.6. Plasma fatty acid (**FA**) profile (µg/mL of plasma) in forage-fed beef cows receiving no supplementation (**NOSUPP**; n = 6 pens), or receiving a molasses-based supplement enriched with Ca salts of soybean oil (24.7% of dry matter; Essentiom, Church and Dwight Co., Inc., Princeton, NJ) via self-fed low-moisture block (**LMB**; n = 6 pens) or hand-fed granular concentrate daily (**CONC**; n = 6 pens). Supplement treatments were provided from d 0 to 42 of the experiment.[1]

Item[3]	**NOSUPP**	**CONC**	**TUB**	**SEM**	***P-value***
Total saturated FA					
d 0	216	218	215	12	0.98
d 14	228[c]	268[b]	308[a]	12	< 0.01
d 28	215[b]	283[a]	267[a]	12	< 0.01
d 42	191[b]	264[a]	270[a]	12	< 0.01
Total monounsaturated FA	52.0	54.9	55.4	2.5	0.60
Total polyunsaturated FA					
d 0	274	267	264	14	0.87
d 14	284[b]	368[b]	458[a]	14	< 0.01
d 28	284[b]	444[a]	440[a]	14	< 0.01
d 42	274[b]	448[a]	474[a]	14	< 0.01
Total ω-3 polyunsaturated FA					
d 0	75.9	74.4	76.3	3.2	0.90
d 14	78.4[b]	55.6[a]	56.5[a]	3.2	< 0.01
d 28	79.5[b]	62.3[a]	61.2[a]	3.2	< 0.01
d 42	73.7[b]	59.0[a]	63.7[a]	3.2	< 0.01
Total ω-6 polyunsaturated FA					
d 0	198	192	188	13	0.84
d 14	205[c]	312[b]	402[a]	13	< 0.01
d 28	204[b]	382[a]	379[a]	13	< 0.01
d 42	201[b]	396[a]	420[a]	13	< 0.01
Total identified FA					
d 0	542[b]	539[a]	536[a]	27	0.98
d 14	568[b]	691[a]	827[a]	27	< 0.01
d 28	550[b]	783[a]	761[a]	27	< 0.01
d 42	515[b]	755[a]	797[a]	27	< 0.01

[1] Blood samples were collected on d 0, 14, 28, and 42 for plasma harvest, and analyzed for fatty acid concentration according to Brandão et al. (2018)

Figure 4.1. Experimental design assigned to beef cows receiving diets supplemented with Ca salts of soybean oil (n = 52) or prilled saturated fat (n = 52) during the last trimester of gestation. Variables include body weight (**BW**), body condition score (**BCS**; Wagner et. al., 1988), blood sample (**BS**), *longissimus* muscle biopsy (**LMB**), and signs of bovine respiratory disease (**BRD**).

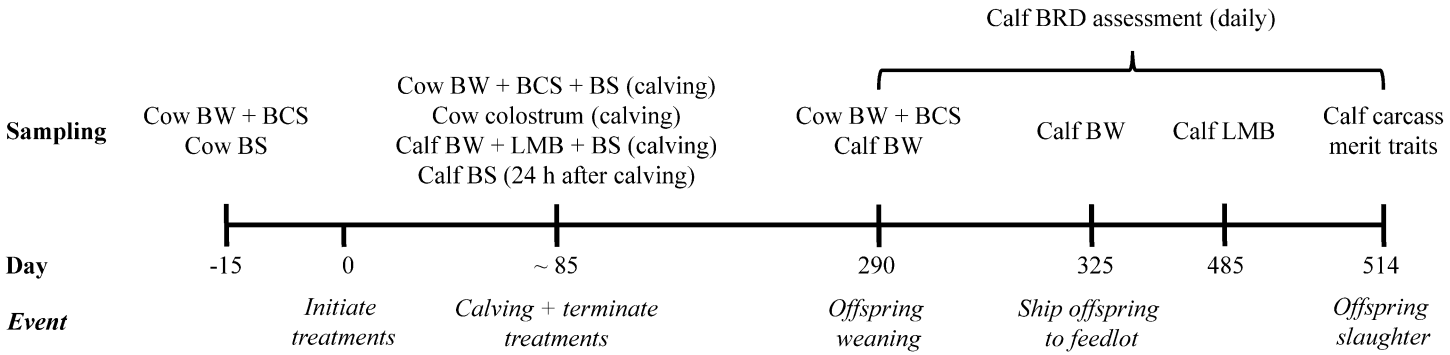

Figure 4.2. Cumulative incidence of bovine respiratory disease (**BRD**), during the initial 3 weeks after feedyard arrival, from the offspring of beef cows receiving diets containing Ca salts of soybean oil (**CSSO**; n = 52) or prilled saturated fat (**CON**; n = 52) during the last trimester of gestation. Cows received (dry matter basis) 195 g/cow daily of CSSO (Essentiom; Church and Dwight Co., Inc., Princeton, NJ) or 170 g/cow daily of prilled saturated fat (Energy Booster 100; Milk Specialties, Eden Prairie, MN). Treatments were provided from d 0 (d 195 of gestation) to calving. Calves were weaned on d 290 of the experiment, preconditioned for 35 d (d 291 to 325), and transferred to a feedyard where they remained until slaughter (d 514). Calves were observed daily for respiratory disease signs based on the DART system (Zoetis, Florham Park, NJ), and received medication according to the management criteria of the commercial feedyard. A treatment × day interaction was detected ($P = 0.03$), whereas no BRD incidence were noted beyond 21 d relative to feedyard arrival. Within days, * $P \leq 0.05$.

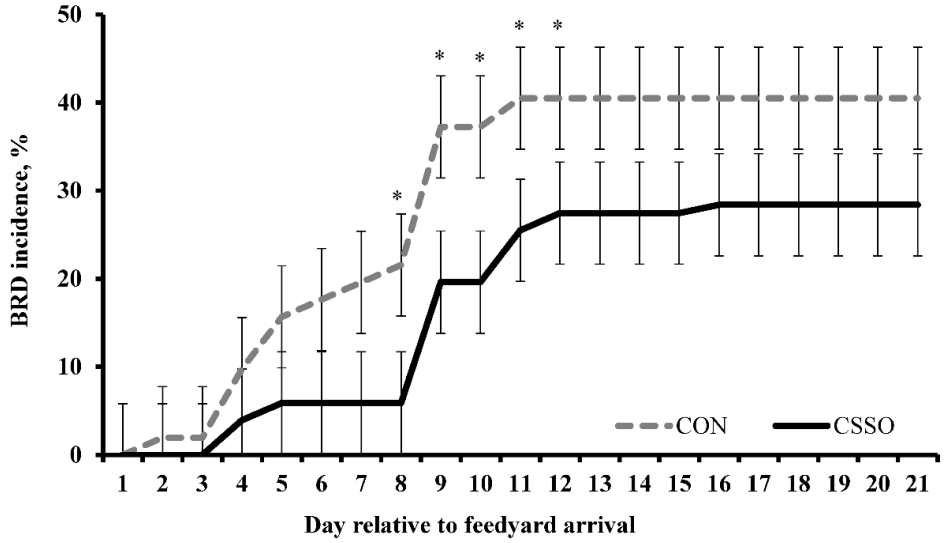

Figure 5.1. Intake of a molasses-based supplement enriched with Ca salts of soybean oil (24.7% of dry matter; Essentiom, Church and Dwight Co., Inc., Princeton, NJ) and delivered to beef cows via self-fed low-moisture block (LMB; n = 6 pens) or via or hand-fed granular concentrate daily (CONC; n = 6 pens). Supplemented treatments were provided from d 0 to 42. The LMB was not weighed from d 0 to 13 to allow cows to adapt and consume blocks without interference from research personnel. The LMB was weighed every other day from d 14 to 42, and divided by 2 to represent daily intake. The CONC was offered at 0.454 kg/cow daily (as-fed basis; 0.420 kg of DM/cow daily) from d 0 to 13, and adjusted (d 14 to 42) in 0.057 kg/cow (as-fed basis) increments/decrements every 2 d to match LMB intake. A treatment × day interaction was detected ($P < 0.01$). Within days; **$P < 0.01$ and *$P \leq 0.05$.