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CONSEQUENCES OF COW-CALF PRODUCTION WITH LIMITED PERENNIAL
FORAGE GRAZING

by

Hannah Fae Speer

A DISSERTATION

Presented to the Faculty of
The Graduate College at the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Doctor of Philosophy

Major: Animal Science
(Ruminant Nutrition)

Under the Supervision of Professor Mary E. Drewnoski

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CONSEQUENCES OF COW-CALF PRODUCTION WITH LIMITED PERENNIAL
FORAGE GRAZING

Hannah Fae Speer, Ph. D.

University of Nebraska, 2023

Advisor: Mary E. Drewnoski

Conversion of perennial grasslands to cropland and rising land prices prompts a need to evaluate alternative beef cow-calf production systems. A 3-yr study was conducted comparing beef cow-calf performance between two August-calving cow systems: perennial forage and corn residue grazing; or summer drylot, fall grazing of late-summer-planted cover crop, and corn residue grazing. Cow body condition score differed between systems, but condition of cows in both systems never fell below adequate. There were no differences in pregnancy rates between systems. Calf weaning weights differed between systems each year; differences are attributed to feedstuff quantity, quality, and type available to calves prior to weaning. Differences in time on green pastures between systems may impact cow supplemental vitamin A needs; therefore, two additional studies were conducted to understand maternal vitamin A supplementation needs in stored forage diets and its impacts on offspring vitamin A status. In the first study, beef cows in mid-gestation were provided supplemental vitamin A below current recommendations for 143 d. Mean cow liver retinol concentration 32 d post-calving was 482 $\mu\text{g/g}$ DM and fell within current adequacy reference ranges, while both calf plasma and liver retinol were below adequate for calves 32 d of age. The second study evaluated the effect of 3 different supplemental vitamin A levels on cow and calf liver retinol concentrations: current recommendation (31,000 IU/d; 1X), 3 times (93,000 IU/d; 3X), or 5 times

(155,000 IU/d; 5X) the current recommendation. Cows receiving 1X maintained liver retinol concentrations below adequate throughout the 165-d supplementation period, whereas cows on 3X and 5X were above adequate by d 81. Calves of 1X cows had lower liver retinol concentrations than 3X and 5X calves. Adequate liver retinol concentrations were only observed in 3X and 5X calves. Overall, cow liver retinol concentrations and dietary vitamin A intake will impact calf vitamin A status. Cows fed stored forage diets may require supplemental vitamin A above current recommendations to ensure adequate status of her and her calf.

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Dedication

In loving memory of Elsie Beth Speer, a true saint that I was blessed to have as a grandma, and Dr. Robert J. Keener, a mentor who was like a second dad to me.

Both have a special place in my heart forever.

CHAPTER 1 - LITERATURE REVIEW

Introduction

Traditionally, cow-calf production has made use of land that is unsuitable for crop production by grazing the forage it provides. The conversion of perennial grasslands to cropland in the Midwest, paired with rising commodity prices, has led to increases in land value and consequently increased pasture rental rates (Wright and Wimberly, 2013; Jansen and Stokes, 2023). In Nebraska, reduced pasture and rising commodity prices have led to record-high land values and pasture rental rates that have nearly doubled over the last decade (Jansen and Stokes, 2023; USDA-NASS, 2023). Widespread drought in the region has also reduced the amount of pasture available for grazing, and in all of these situations, it creates a need for evaluating alternative cow-calf production strategies that require less perennial pasture acres. Corn residue, an inexpensive forage resource, can be used for fall/winter grazing in areas with limited pasture but an abundance of acres planted to corn (Klopfenstein et al., 1987). Confinement feeding of cows is strategy than can be used to maintain the cow-calf herd when traditional grazing is not an option. Fortunately, cow-calf producers in the Midwest have access to feeds that can be combined to create diets that meet the nutritional needs of their cow herd in confinement. For example, by-products generated from the ethanol industry, like distillers grains, and low-quality forages such as crop residues, can be combined to form a low-cost ration that meets the nutrient requirements of cows (Shike et al., 2009; Jenkins et al., 2015; Meter et al., 2018).

One challenge of confinement cow systems, or any cow-calf production system operating with less access to fresh green forage, is the lack of β -carotene, a natural source

of vitamin A, in the diets. Generally, cows who have the ability to graze green summer pastures are not at risk of developing a vitamin A deficiency because they are consuming a large amount of β -carotene. Beta carotene is a provitamin A carotenoid, meaning it can be used by the body to make vitamin A. Any vitamin A made in excess can then be stored in the liver for times when dietary vitamin A intake may be low. Herds relying on stored forages, crop residues, and grains/grain by-products to make up the majority of their diet may be at risk of vitamin A deficiency due to the lack of β -carotene found in these ingredients (Pickworth et al., 2012). Current NASEM (2016) recommendations for supplemental vitamin A may not be appropriate for the cow managed in a system with little to no access to green pasture throughout the year because she is not given the opportunity to build liver stores of vitamin A, and she is receiving very little from her diet. Recommendations for supplemental vitamin A have not been changed since the 1970s (NRC, 1976), due in large part to the fact that no new research has been conducted in this area. Of the studies cited behind this recommendation, only three were conducted with beef cows (Guilbert and Hart, 1935; Church et al., 1956; Meacham et al., 1970)

The purpose of the following review will be to cover basic concepts of vitamin A nutrition, evaluation of vitamin A status in beef cattle, and current knowledge surrounding vitamin A requirements of beef cows and their calves.

Overview of properties, metabolism, and function of vitamin A

Before discussing vitamin A in the context of cow-calf systems, it is important to understand some basics of vitamin A metabolism and its role in various bodily functions and biological processes. Vitamin A is a fat-soluble vitamin, and the term vitamin A

refers to any compound that shares similar biological activity to retinol (Goodman, 1984; Blomhoff et al., 1992). It cannot be made in the body; therefore, it must be obtained from diet or supplementation. Vitamin A can be obtained from both animal and plant sources. Animal sources provide preformed vitamin A in the form of retinyl esters. Plant sources, on the other hand, contain provitamin A carotenoids, such as β -carotene, which can be converted into active forms of vitamin A in the body.

Forms and function of vitamin A

Vitamin A exists in several forms, and each form has a unique function. Retinol is the alcohol form of vitamin A, and is the form found in circulation for transport around the body for use in other tissues. It is bound to retinol-binding protein (RBP), a carrier protein designed to protect retinol from oxidation during transport to other tissues (Combs, 2012). Once retinol is taken into a cell, it has one of three fates: 1) conversion to retinyl esters (storage form) and stored; 2) converted to another active form of vitamin A (retinal or retinoic acid); or 3) catabolized and subsequently excreted from the body (Blomhoff et al., 1992).

Retinal is an intermediate form of vitamin A that is involved in the visual cycle and is essential for proper vision. In rod cells, retinal is bound to opsin to form rhodopsin, a pigment (visual purple) involved in the process of vision (Linus Pauling Institute, 2021). It helps maintain good vision, particularly in low light conditions. Light absorbed by the rod cell catalyzes the rapid isomerization of 11-*cis*-retinal to all-*trans*-retinal and its subsequent release from opsin (Combs, 2012; Linus Pauling Institute, 2021). This process triggers a cascade of events that result in the generation of a nerve impulse

conveyed by the optic nerve to the vision center of the brain (Combs, 2012; Linus Pauling Institute, 2021). The all-*trans*-retinal is converted to all-*trans*-retinol and transferred back to the retinal epithelial cell where it is stored in the lipid fractions of the cell until it is ready to be used again in the visual cycle (Combs, 2012; Linus Pauling Institute, 2021).

Retinoic acid is the form of vitamin A involved in gene expression and regulating cell growth and differentiation of various tissues, including skin, bone, immune, and epithelial cells. It is able to regulate the expression of over 500 retinoid-responsive genes by binding to nuclear receptor proteins: retinoic acid receptors (RAR) and retinoid X receptor (RXR) that form heterodimers (RAR/RXR) or homodimers (RAR/RAR or RXR/RXR) that then bind to retinoic acid responsive element (RARE) in the promoter region of target genes, thereby controlling the transcription of genes (Blomhoff et al., 1990; Semba, 1998; Linus Pauling Institute, 2021).

General functions of vitamin A

The role of vitamin A in the immune system is far-reaching, and has been reviewed by Semba (1998). Briefly, the author describes that vitamin A supports the immune system by maintaining the integrity of epithelial cells which act as a barrier against pathogens, especially in the gastrointestinal and respiratory tracts. It also plays a role in the production and function of immune cells in both the innate and adaptive immune systems (Semba, 1998). In young calves, Nonnecke et al. (1999) demonstrated that leukocyte populations at 7 wk of age in calves receiving 32,000 IU/d of supplemental

vitamin A were similar to those found in adult cattle, suggesting that vitamin A supplementation to calves could aid in immune system maturation.

Vitamin A is important for reproductive health in both males and females. During pregnancy, it is essential for proper embryonic development and organ formation. The role of vitamin A in female reproduction varies from the time of conception to birth. In a recent review, Clagett-Dame and Knutson (2011) described the role of vitamin A in maintaining epithelial tissue health in the reproductive tract prior to mating. Vitamin A status of the cow during gestation is important because of its role in embryonic and fetal development and placental growth (Takahashi et al., 1977; Blomhoff et al., 1990; Clagett-Dame and Knutson, 2011). Deficiencies could result in complete failure to breed, fetal resorption, abortion, or congenital malformations; thus, the time point at which a vitamin A deficiency is present will affect reproductive outcome (Clagett-Dame and Knutson, 2011). In bulls, the role of vitamin A appears to be important for maintaining sperm concentration, semen volume, and reducing the amount of abnormal sperm (Roussel et al., 1963).

Vitamin A metabolism

Absorption of vitamin A and carotenoids primarily occurs in the small intestine along with dietary fats. In the intestinal lumen, bile salts and pancreatic enzymes aid in releasing carotenoids and retinyl esters from food matrices, and subsequently package free carotenoids and free retinol into micelles for absorption into enterocytes (Combs, 2012). Once absorbed, these compounds can be processed further. Retinol can be re-esterified and be packaged into chylomicrons along with carotenoids to enter the lymph

system (Blomhoff, 1994). Beta-carotene can be cleaved into 2 retinals by the enzyme β -carotene 15,15'-dioxygenase, and retinal can either be reduced to retinol or oxidized into retinoic acid (Goodman, 1984; Blomhoff et al., 1992; Combs, 2012). Differences in activity of this enzyme exist across species, and consequently, conversion efficiency of β -carotene to retinol is different across species (McDowell, 2000). Cattle are not considered to be efficient converters of β -carotene, and have only 24% of the conversion rate of rats, which is considered to be the standard conversion rate that all other species are compared to (McDowell, 2000). Interestingly, differences in conversion efficiency and absorption have been found in dairy breeds. Holsteins are more efficient converters compared to the Jersey and Guernsey breeds, who absorb more β -carotene intact (Frye et al., 1991; McDowell, 2000). It has been found in rats that β -carotene 15,15'-dioxygenase activity is upregulated in times when dietary vitamin A intake is low, and is lower when β -carotene or vitamin A intake is high (van Vliet et al., 1996), suggesting there is a regulatory mechanism in place to help prevent excess vitamin A in circulation and accumulation in the liver.

Once absorbed, retinyl esters and carotenoids are secreted from the enterocytes packaged in the chylomicrons and transported to the liver for uptake via the lymph system. The liver takes up retinyl esters and hydrolyzes them to retinol in parenchymal cells, and the retinol is transferred by retinol-binding protein to stellate cells where it is re-esterified and stored in the liver as retinyl esters, specifically as retinyl palmitate, until it is needed by the body (Combs, 2012). Beta carotene may either be cleaved to retinal, incorporated into VLDL, or stored in hepatic cells, but its exact fate once it reaches the liver is not well-known (Bohn et al., 2019). Lecithin-retinol acyltransferase (LRAT), an

enzyme in the liver that esterifies retinol, seems to be regulated by vitamin A status. Its activity is minimal in the case of vitamin A deficiency, but when vitamin A intakes are adequate, its activity increases (Ross and Zolfaghari, 2004). This regulation is reported to be controlled by retinoic acid, which regulates LRAT gene expression in the liver (Ross and Zolfaghari, 2004). When vitamin A is mobilized from the liver, the retinyl esters are hydrolyzed and retinol is bound to RBP and transported to peripheral tissues (Blomhoff et al., 1992; Combs, 2012).

Vitamin A deficiency

The various roles that vitamin A plays in the body means there are numerous ways a vitamin A deficiency can present itself. Two major categories vitamin A deficiency impacts are vision and processes of cellular growth and differentiation (DSM, 2021). The one deficiency symptom that is unique to vitamin A deficiency is night blindness; rhodopsin is not able to form in the absence of retinal. Because of retinoic acid's involvement with gene expression, tissues in many places throughout the body are affected. Epithelial cells that line the eye, gastrointestinal, respiratory, and urogenital tracts are unable to grow and differentiate properly, which results in keratinization (Semba, 1998; McDowell, 2000). The compromised barrier in these tracts makes an individual more susceptible to infection because pathogens are able to pass easier into the animal's system. This is especially evident in young calves. One of the most commonly reported symptoms associated with vitamin A deficiency in calves is diarrhea and/or respiratory infections in the first week or two of life (Guilbert and Hart, 1934; Stewart and McCallum, 1938; Church et al., 1956; Jones et al., 1962). Because of vitamin A

deficiency, their immune system is also producing less immune cells, and the activity of some of those immune cells is reduced (Semba, 1998; McGill et al., 2019)

While a deficiency can be recognized by presentation of one or more of these symptoms, other diagnostic methods are available for detecting a deficiency before it reaches clinical stages. The following section will discuss methods for evaluating vitamin A status and acceptable reference ranges for the indices discussed.

Indices of vitamin A status

The two biomarkers utilized to evaluate vitamin A status in cattle most commonly are plasma and liver retinol concentrations. The easiest and least invasive sample to collect is blood, but plasma retinol concentration is not a very sensitive indicator of vitamin A status. Circulating retinol levels are tightly regulated by homeostatic control mechanisms, and will not fluctuate (Blomhoff et al., 1992; Goodman, 1984) unless liver stores become severely depleted (Dowling and Wald, 1958; Olson, 1984) or when liver storage capacity is exceeded (Olson, 1984). Therefore, the extent to which plasma retinol is useful in assessing vitamin A status is identifying if a clinical deficiency or a toxicity is present. Plasma retinol becomes even less useful as a biomarker of vitamin A status in gestating cows. Cow plasma concentrations of vitamin A have been noted to decrease from late gestation to a nadir at parturition, then begin to gradually increase in the early weeks of lactation (Johnston and Chew, 1984; Goff and Stabel, 1990; Oldham et al., 1991; Chawla and Kaur, 2004; Puvogel et al., 2008). When evaluating the relationship between peripartum plasma retinol concentrations and mastitis infection in dairy cows, Johnston and Chew (1984) reported a sharp decline in plasma retinol concentrations in

both mastitic and nonmastitic cows from 7 d prepartum to time of calving. Oldham et al. (1991) observed a decrease in serum vitamin A concentrations in dairy cows about 3 months prior to calving, regardless of level of supplemental vitamin A included in the diet. The decline in plasma retinol prior to parturition has been attributed to the transfer of retinol into colostrum (Goff and Stabel, 1990; Goff et al., 2002). This was demonstrated by Goff et al. (2002) with mastectomized periparturient dairy cows, whose plasma retinol concentrations did not decrease 14 d before parturition. The reduction in plasma retinol concentrations before calving appears to be a normal physiological occurrence rather than a sign of deficiency. These studies provide additional evidence that plasma concentrations of vitamin A are a poor reflection of vitamin A status because perceived vitamin A status based on plasma retinol concentration can change with stage of production.

The homeostatic regulation of plasma retinol also means that it is not sensitive to changes in dietary vitamin A intake. Oldham et al. (1991) provided dairy cows with 50,000 or 170,000 IU/d of vitamin A as retinyl acetate (a retinyl ester), or 170,000 IU/d as both retinyl acetate and β -carotene, approximately 75 d before calving. Plasma retinol concentrations did not differ between treatments at any point in those 75 d, but did decline up until parturition. In a study with feedlot steers supplemented with either 0, 55, 220, 440, or 1100 IU/kg BW, plasma retinol concentrations at d 277 of supplementation did not differ significantly between the 3 higher levels of supplementation (Frey et al., 1947). The plasma retinol concentration observed at these higher levels of supplementation was around 500 ng/mL. Conversely, the liver retinol concentrations of these steers appeared to increase with increasing amounts of vitamin A supplementation

in a near-linear fashion, suggesting that liver retinol concentrations are a sensitive indicator of dietary vitamin A intake, and therefore vitamin A status. Approximately 90% of total body vitamin A is stored in the liver (Olson, 1984; McDowell, 2000). Liver samples are more reflective of changes in dietary vitamin A intake, as they increase when vitamin A intake exceeds physiological need, or decrease when vitamin A intake is insufficient to meet the body's needs (Olson, 1984). At the time of this writing, it is unclear how liver vitamin A concentration changes with stage of production. One study noted in spring-calving range cows that liver concentrations could peak during months when green forage was available and decrease in subsequent months (Wheeler et al., 1957). The peak of liver stores occurred when cows would be expected to be in peak lactation. In this instance, it appears that fluctuation in liver concentration throughout the production cycle may be confounded by dietary intake of vitamin A, which makes it difficult to definitively say what is causing those fluctuations.

Reference ranges for indices of vitamin A status

The concentration of vitamin A in liver and plasma considered adequate for mature cattle is 300 $\mu\text{g/g}$ DM and 300 ng/mL in plasma, respectively (Puls, 1994). It is unclear how these reference values were obtained, and there may be some margin of safety applied. Church et al. (1956) observed no symptoms of vitamin A deficiency in cows that had liver vitamin A concentrations ranging from 16 to 153 $\mu\text{g/g}$ DM and plasma vitamin A concentrations ranging from 150 to 260 ng/mL at 12 weeks into lactation. Others have observed symptoms of abortion, calves born prematurely, and night blindness in cows when liver vitamin A concentrations were less than 5 $\mu\text{g/g}$ DM

(Wheeler et al., 1957; Swanson et al., 1968), so it appears that cow liver stores must be severely depleted before clinical symptoms appear.

Current reference values for liver and plasma vitamin A indicate that concentrations of 100 $\mu\text{g/g}$ DM and 225 ng/mL in liver and plasma, respectively, are considered adequate (Puls, 1994) for calves around 30 d of age. However, other data suggests that these thresholds may overestimate adequate concentrations. Thomas and Moore (1952) indicated that at liver vitamin A concentrations in calves approximately 4 to 6 mo of age less than 2.1 $\mu\text{g/g}$ on a wet basis (7 $\mu\text{g/g}$ DM; assuming 30% DM content of liver), plasma vitamin A concentrations were decreased. Calves with liver vitamin A concentrations this low could be considered clinically deficient (Puls, 1994); however, authors did not report any health information on these calves. Another study conducted with dairy calves observed only 5 cases of mild diarrhea (out of 28 calves) from birth to 28 d of age with liver vitamin A levels at 28 d of age ranging from 60 to 80 $\mu\text{g/g}$ DM, and plasma vitamin A concentrations of 150 ng/mL (Nezvesky et al., 1950). Swanson et al. (2000) observed less incidence of diarrhea and hyperthermic rectal temperatures in dairy calves from d 4 to 28 of age when supplemental vitamin A level increased from 2,300 IU to 44,000 IU/kg DM. When concentrations of less than 9,000 IU/kg DM were provided, decreases in liver and plasma retinol concentrations were observed, while calves receiving 9,000 IU/kg DM had essentially maintained liver and plasma retinol concentrations at 75 $\mu\text{g/g}$ DM and 94 ng/mL , respectively, through d 28. These studies suggest that perhaps the threshold before seeing adverse health effects in young calves may be lower than retinol concentrations of 100 $\mu\text{g/g}$ DM and 225 ng/mL in liver and plasma, respectively.

Vitamin A in feedstuffs

The plant-based nature of cattle diets means cattle consume vitamin A in their diet in the form of provitamin A carotenoids. Carotenoids are what give a plant its red (lycopene), yellow (xanthophylls), or orange (carotenes) pigments. There are over 600 naturally occurring carotenoids; however, less than 10 are found in feedstuffs commonly included in cattle diets (Noziere et al., 2006). Not all carotenoids have provitamin A activity, meaning not all can be converted into active forms of vitamin A. This is due to the differences in structures of the carotenoids; anything containing a β -ionone ring can be transformed into vitamin A, while those lacking this cannot. The provitamin A carotenoids of significance found in feedstuffs fed to cattle would be α -carotene, β -carotene, γ -carotene, β -cryptoxanthin (Pickworth et al., 2012).

Forages

Vitamin A content of feedstuffs that are commonly fed to beef cattle are displayed in Table 1.1 (Pickworth et al., 2012). Generally, cattle that are grazing lush green pasture during the spring and summer months will not experience a vitamin A deficiency, as the amount of β -carotene is sizable in fresh green forages (Puls, 1994; Weiss, 1998; Pickworth et al., 2012). In a recent analysis of commonly utilized feedstuffs in the U.S. in beef cattle rations, Pickworth et al. (2012) concluded carotenoid content from forages was dependent on growing conditions, maturity at harvest, storage conditions, and processing methods. Harvesting and storing forages for prolonged periods of time will greatly reduce vitamin A content, as factors such as light, heat, and moisture greatly

reduce vitamin A content (Puls, 1994; Ballet et al., 2000). Wheat straw, due to its lack of green color, is practically devoid of vitamin A (Table 1.1). For example, corn silage appears to be an excellent source of vitamin A; however, significant depletion of liver vitamin A stores has been noted with steers consuming corn silage (Jordan et al., 1963; Smith et al., 1964). This is likely due to β -carotene losses that can occur during ensiling (Puls, 1994). Losses can occur during wilting prior to ensiling, higher pH, and delays in sealing the silo (Kalac and McDonald, 1981; Noziere, 2006).

Grains and concentrates

Grains and grain by-products generally contain low levels of provitamin A carotenoids in comparison to forages (Pickworth et al., 2012). One exception to this is corn; because of its yellow color, it contains considerable amounts of provitamin A carotenoids (Table 1.1). Wet and dry distillers grains also contain appreciable levels of vitamin A activity, with wet being higher in vitamin A activity compared to dry (Table 1.1)

Relationship between cow and calf vitamin A status

Calves are born deficient in vitamin A because placental transfer of fat-soluble vitamins is minimal (Malone, 1975). Colostrum is a good source of vitamin A as evidenced by studies indicating increased plasma vitamin A concentrations of calves after colostrum ingestion shortly after birth (Zanker et al., 2000; Puvogel et al., 2008). Dietary vitamin A levels the cow receives during late gestation, as well as her liver vitamin A stores, affect the amount of vitamin A her calf receives via colostrum to build its own

liver vitamin A stores. In calves at 28 d of age, Nezvesky et al. (1950) observed greater liver vitamin A concentrations in calves fed colostrum from cows supplemented 30 d before calving with 1,000,000 IU/d vitamin A compared to calves receiving colostrum from cows receiving no vitamin A supplement. Research in beef cattle indicates cow stores only contribute about 40% of the vitamin A found in colostrum, while the other 60% comes from the cow's diet (Branstetter et al., 1973; Tomlinson et al., 1974). Cows used in the research to figure out these contributions were supplemented 45,000 IU/d (Branstetter et al., 1973) or 50,000 IU/d (Tomlinson et al., 1974), in diets consisting of primarily alfalfa-grass hay supplemented with corn. Liver vitamin A concentrations in these studies were reported to be maintained at a minimum of 200 $\mu\text{g/g}$ (unclear if on a wet or dry basis). At this time, there is no suggested amount that should be supplemented to the beef cow that will ensure both adequate status in both her and her calf.

Vitamin A requirements of beef cows

When discussing a requirement for any nutrient, it is important to understand how its requirement is defined. Nutrient requirements can be characterized as either a physiological requirement or a dietary requirement. The former requirement refers to the amount and form of the nutrient an individual needs to support physiological processes such as maintenance, growth, pregnancy, and lactation; the latter requirement is how much of the nutrient needs to be consumed to meet the physiological requirement (Iglesia et al., 2010). Physiological and dietary requirements are often not distinguished from one another, and often interpreted as one in the same. It is erroneous to assume they are both

the same as digestion, metabolism, and other dietary factors can change the bioavailability of the nutrient versus what the diet was targeted to provide.

True vitamin A requirements of cattle are often difficult to determine because factors like environment, composition of the diet, genetics, stage of production, and physiological processes can influence requirements (McDowell, 2000). It is also worth mentioning that vitamin A requirements can vary based on the criteria used to establish them. For example, a requirement for preventing clinical deficiency can be different than what is required to for successful reproductive function (Guilbert et al., 1940). The word “requirement” in the context of vitamin A requirements of beef cows gives the impression there is sufficient information available being used to confidently generate a requirement, when in reality, it is a topic that has not been well-researched. In fact, the dairy NASEM (2021) describes vitamin A needs of cows as an adequate intake for supplemental vitamin A rather than an absolute requirement for total vitamin A. They do this because there is a limited number of studies assessing vitamin A requirements of dairy cattle, and variability in responses across studies due to differences among cattle within each study. Thus, in this review, the term “requirement” will be replaced with recommendation, as there is much that is still unknown about vitamin A needs of beef cows.

Review of studies used to establish vitamin A recommendations

The few studies that the vitamin A recommendations for beef cows are founded on were conducted over 50 years ago, and of those studies, only 3 (Guilbert and Hart, 1935; Church et al., 1956; Meacham et al., 1970) were conducted with beef cows. These

studies will be reviewed in order to understand how results obtained translate to the current vitamin A recommendations of beef cows.

Guilbert and Hart (1935) briefly summarized observational data collected on 7 gestating beef heifers from a previous study (Guilbert and Hart, 1934) fed diets essentially devoid of vitamin A to deplete their liver stores. Two heifers received the basal diet of dried beet pulp and wheat straw, and the rest of the heifers were supplemented with alfalfa hay as a source of carotene. Two of the heifers receiving the basal diet and alfalfa hay showed no signs of deficiency, but their calves developed night blindness (60 days of age) and/or diarrhea (7 to 60 days of age) and symptoms disappeared after treatment with cod liver oil, which is a source of vitamin A. In fact, all five calves born in this study (Guilbert and Hart, 1934) developed severe diarrhea within the first few weeks of life: one died at 6 d of age following treatment with cod liver oil for 3 d, and one recovered without treatment.

Subsequent experiments utilizing all but 1 heifer from their previous study (Guilbert and Hart, 1934) along with 4 additional animals (at least 1 bull) supplemented alfalfa hay or dehydrated alfalfa meal to provide β -carotene and suggested 26 to 33 μg carotene/kg BW as a minimum requirement for preventing night blindness in cattle (Guilbert and Hart, 1935). This minimum recommendation equates to 10.4 to 13.2 IU/kg BW as 1000 μg of β -carotene is equal to 400 IU of vitamin A (McDowell, 2000). When discussing the requirement for reproduction, Guilbert and Hart (1935) noted that females were not able to produce a healthy calf when fed carotene deficient rations long-term and provided carotene supplementation in the form of alfalfa hay, which provided an additional 9.5 to 15 mg of carotene (3,800 to 6,000 IU) per day to their diet. These early

studies suggest that minimum vitamin A requirements for the cow are not able to prevent vitamin A deficiency in the young calf.

Meacham et al. (1970) conducted two experiments where gestating beef cows were supplemented 16,000 IU/d to a grass silage and hay-based diet for either 55 (Exp. 1; 255 cows supplemented) or 106 d (Exp. 2; 157 cows supplemented) prior to calving, and then 40,000 IU/d during lactation. The basal diet was reported to have the equivalent of approximately 5,000 IU/kg, meaning cows were consuming an additional 46,000 and 72,000 IU/d from the basal diet in gestation and lactation, respectively. The BW of the cows was not reported, but if cow BW is assumed to be 478 kg based on historical BW of cows during the 1970's (McMurry, 2009), total vitamin A intake during gestation would have equated to 96 and 130 IU/kg BW for the non-supplemented and supplemented treatments, respectively. The supplement amount of 16,000 IU/d alone would have provided 29 IU/kg BW, which is nearly triple the amount suggested by Guilbert and Hart (1935). When supplementation in gestation was 55 d, death losses of calves in experiment 1 were 16.5% in the non-supplemented group and were almost double that of the supplemented group (8.8%). However, in trial 2, when they moved the calving season from January to March and extended the period of supplementation during gestation, death losses of calves was minimal and not different between treatments (mean 3%). This occurred even though the cows in the supplemented and non-supplemented groups in experiment 2 had greater loss of and lower overall vitamin A reserves at the end of the experiment than the non-supplemented cows in experiment 1. Essentially, supplementation of 16,000 IU/d in the experiments was not enough to prevent a reduction in vitamin A liver stores whether supplemented to cows 55 or 106 d prior to calving. The

lower death loss was attributed to less adverse weather conditions with the later calving season; however, it is possible that extending supplementation from 55 to 106 d before calving could have impacted the amount of vitamin A in colostrum while not being sufficient to maintain cow liver reserves.

It is not apparent how the NRC (1976) utilized the vitamin A studies it cited conducted with beef cows to set vitamin A recommendations. Guilbert and Hart (1935) suggest 26 to 33 μg carotene/kg BW is sufficient to prevent clinical deficiency in cattle, but almost five times this minimum carotene level (125 μg carotene/kg BW) is recommended to ensure successful reproduction and birth of a healthy calf, as well as build liver vitamin A stores (Guilbert et al., 1940). Again, this minimum recommendation equates to 10.4 to 13.2 IU/kg BW, and the recommendation for reproduction (Guilbert et al., 1940) would be 50 IU/kg BW. A 591-kg cow's requirement, then, would be 29,550 IU/d, assuming the latter number better represents the vitamin A needs of a non-lactating beef cow if reproductive success is the goal. If these values are now expressed in IU/kg DM, this would mean she would need 2,273 IU/kg DM (estimated DMI = 2.2% BW). This is slightly lower than the current recommendation of 2,800 IU/kg DM for gestating cows (NASEM, 2016), but it is not known if the preceding calculations are how the NRC (1976) arrived at this number. In the study of Meacham et al. (1970), the basal diet alone provided almost double the current recommendation for gestating beef cows (NASEM, 2016) at 5,000 IU/kg DM. When trying to understand the thought process behind how current vitamin A recommendations for beef cows were established, the two studies that used beef cows cited behind the 2,800 IU/kg DM recommendation (NASEM, 2016) do not provide much insight into why this supplemental level is suggested.

Currently established lactating cow recommendations are even less clear than those for gestating cows. The one study evaluating supplementation of vitamin A to lactating beef cows utilized 48 cows and only compared two different levels of supplementation (Church et al., 1956). Cows used in this study were 3-yr old Herefords, who entered the study at an average BW of 340 kg. All cows were provided with a vitamin A-deficient (i.e., low β -carotene) ration consisting of ad libitum access to low quality hay and a concentrate supplement of cottonseed meal starting in gestation. Rolled milo was added to the concentrate mix during lactation, and cows were provided either a low or high level of carotene supplement at this time. The amounts fed of the concentrate mix were 1.67 kg/d. The low level of carotene was 27 mg/d (10,800 IU/d) and the high level was 126 mg/d (50,400 IU/d). Based on mean BW of the cows at the start of the study, authors reported these levels to range from 0.08 to 0.37 mg carotene (32 to 148 IU) per kg BW. They observed that the high level of supplementation was not enough to prevent loss of liver vitamin A stores over 12 wk of lactation. Furthermore, liver vitamin A concentrations at the end of lactation were less than 200 μ g/g on a DM basis for cows receiving the high level of supplementation, a concentration indicative of a marginal deficiency (Puls, 1994). Calf liver vitamin A concentrations at 12 wk of age were reported to be 1.5 to 3.6 μ g/g DM, which is extremely low and indicates a clinical deficiency (Puls, 1994). Indeed, clinical symptoms were present in some calves, regardless of the supplemental amount their mothers received during lactation. Authors reported calves to have diarrhea, excess lacrimation, and excessive dryness and inflammation of the eyes in calves that survived to 12 wk of age. Any calves that died before the end of the experiment were reported to have died from combinations of

vitamin A deficiency, malnutrition, and secondary infections. The highest level of supplementation in this study (148 IU/kg BW) provided 50,320 IU/d to cows in lactation. If you compare it to the current NASEM (2016) recommendation for lactating cows (3,900 IU/kg), assuming a 591-kg lactating cow with a DM intake of 2.2% of her BW, or 13 kg per day, this would be 50,700 IU/d. However, if the 148 IU/kg BW is translated to the BW of the 591-kg lactating cow, the high supplemental level used by Church et al. (1956) would be 87,468 IU/d, or 6,278 IU/kg DM. Similar to current gestating beef cow requirements, it is not clear how the current lactation requirement was arrived at for beef cows based on this study.

Current vitamin A recommendations of beef cows

Dietary amounts of vitamin A and recommendations are expressed on an international unit (IU) basis. Different dietary sources have different amounts of vitamin A activity. For cattle diets, 1 IU is equivalent to 2.5 μg of β -carotene, 0.3 μg of all-*trans* retinol, 0.344 μg all-*trans* retinyl acetate, or 0.550 μg all-*trans* retinyl palmitate (NASEM, 2021). Current NASEM (2016) vitamin A recommendations for pregnant and lactating beef cows are 2,800 IU/kg and 3,900 IU/kg DM of feed, respectively. These recommendations are for supplemental vitamin A only, meaning that these are recommended feeding levels above what vitamin A is already being contributed by the diet. It is not entirely clear what type of diet, if any, was considered when first setting these recommendations in the NRC (1976). It is assumed NASEM (2016) recommendations were established based on preventing a clinical deficiency for the cow

managed in a pasture-based system, and that this cow has adequate vitamin A liver stores to pull from in times where dietary vitamin A intake is low.

The fact that diet type is not specified is one concern that arises with these recommendations. In the dairy world, vitamin A recommendations are provided with consideration to diet type, and they assume that dairy cows are being fed stored forages and concentrates (NRC, 2001; NASEM, 2021). Composition of the diet is important because fresh green pasture or forages will provide a lot of β -carotene (Pickworth et al., 2012), a provitamin A carotenoid, which can be converted to vitamin A that is either used right away or stored by the cow in her liver for later use. Carotenoids are an essential part of photosynthesis, a process that occurs in the green tissue of plants (Sun et al., 2022). Thus, the green color of a forage is a good indicator of β -carotene content, meaning the amount of vitamin A supplied by the diet is largely tied to forage quality. If current (NASEM, 2016) recommendations were in fact based off of the cow grazing green pastures for a large part of the year, these recommendations may not be appropriate for beef cows who are consuming more harvested than fresh forages in their diet.

Another problem with the recommendations, which is acknowledged by NASEM (2016), is that they do not take into consideration genetics of today's beef cows. Genetic selection for growth and performance over time has resulted in the modern beef cow being significantly larger than cows at the time the studies assessing vitamin A requirements in beef cows were conducted (between the 1930s and 1970s). For example, the Hereford cows used in the study of Church et al. (1956) were approximately 3 years old and weighed only 341 kg at the initiation of experiment. Cow size is not specified in the studies of Guilbert and Hart (1935) and Meacham et al. (1970); however, it is safe to

assume that they too are a much different size compared to present-day beef cows. In the grand scheme of things, changes in cow size is probably not an issue if the recommendations are set on IU/kg DM or IU/kg BW basis.

The final critique of current (NASEM, 2016) recommendations is that they do not consider the vitamin A needs of the offspring. Young calves are born deficient in vitamin A because there is minimal placental transfer of this vitamin in utero (Malone, 1975). They depend on colostrum at birth to provide them with vitamin A, which has much greater concentrations of vitamin A in comparison to whole milk (NRC, 2021). Dietary vitamin A intake of the cow during gestation can influence the amount found in colostrum (Schweigert, 1990; Kumagai et al., 2001; Puvogel et al., 2008), and can thus have impacts on vitamin A status of the young calf. The review of the studies cited used to set the current vitamin A recommendation illustrates that meeting the vitamin A needs of the cow does not mean that the calf's vitamin A needs will also be met.

Comparison of vitamin A recommendations

Vitamin A recommendations for cows across publications are not in agreement with one another. This is due to differences in production goals, performance measures, as well as diet type. For example, vitamin A requirements for dairy cattle (NRC, 2001) were set assuming cows would be receiving a diet consisting primarily of stored forages. Diet type is not specified in other currently established vitamin A requirements of beef cows (CSIRO, 2007; NASEM, 2016). In the case of dairy cattle, it is likely that the requirements were set with a focus on cow performance and less focused on offspring

since the dairy cow and calf are managed separately following parturition. Dairy (NRC, 2001) and beef (NASEM, 2016) recommendations are supplemental recommendations, while other publication like CSIRO (2007) and the small ruminant NRC (2007) make a recommendation for what appears to be the total vitamin A needed.

For comparison, we will refer back to the 591-kg cow consuming 2.2% of her BW in DM per day, and compare recommendations for gestating cows. Based on current NASEM (2016) recommendations, her supplemental vitamin A need would be 33,000 IU/d. For this same cow, the dairy NRC (2001) would say she needs 65,000 IU/d of supplemental vitamin A. The total amount of vitamin A as suggested by CSIRO (2007) would be around 89,000 IU/d. The higher recommendation is accounting for the additional functions that vitamin A has in the body (CSIRO, 2007). Lastly, if we scale the small ruminant vitamin A recommendation (NRC, 2007) for a ewe in late gestation to the 591-kg cow, it would be almost 90,000 IU/d. This recommendation accounts for increased growth of conceptus during late gestation as well as the increased maintenance requirements of animals during late gestation (NRC, 2007). Again, it is unclear if CSIRO (2007) and the small ruminant NRC (2007) are total or supplemental vitamin A recommendations; however, they may be accounting for additional factors in their recommendations that should be considered in vitamin A recommendations for beef cows.

Vitamin A supplementation

Vitamin A supplementation will be warranted when cows are consuming stored forages and concentrates. As previously discussed, this is because these feedstuffs are

low in β -carotene. Current vitamin A recommendations are likely adequate if cows are consuming this diet over a 3 to 4 month period (NASEM, 2016) and have good vitamin A liver stores beforehand. Supplemental vitamin A needs may be increased above what NASEM (2016) suggests in situations where the forage is low quality and brown. Needs for supplemental vitamin A are further increased in situations where the cows are in long-term confinement receiving rations comprised of stored feedstuffs because this type of diet does not allow for liver vitamin A stores to build, which results in less stores for the cows to pull from to meet vitamin A needs. Low vitamin A stores are also a problem in situations where the time spent on green grass is cut short, like with drought. Lastly, anytime the immune system is challenged it may further increase their supplemental requirement because vitamin A plays a large part in immune system function, and will significantly decrease liver stores. A challenge to the immune system could be anything from disease to consuming high levels of toxic compounds in feed such as mycotoxins. For example, vitamin A liver stores in unvaccinated dairy calves who were deemed to have adequate status experienced a significant decrease in liver vitamin A stores when challenged with BRSV (McGill et al., 2019). There seemed to be an overall loss in liver vitamin A stores, and authors were unsure if this was because vitamin A was being excreted during the course of the infection or irreversibly utilized by the immune system.

In earlier literature discussing vitamin A supplementation to beef cattle, the supplemental vitamin A was often in forms that were often unstable. Today, technology has significantly improved and much more stable forms of vitamin A have been created to withstand a variety of conditions such as moisture, heat, and pH. The most common form utilized to supplement beef cattle is the cross-linked beadlet, which is the type

commonly found in vitamin and mineral premixes. Vitamin A, as retinyl acetate, is encapsulated in a starch-protein matrix to create the beadlet. The starch-protein matrix protects the retinyl acetate from moisture and pH, and is insoluble in water (DSM, 2023). Retinyl acetate is esterified retinol, a chemical modification that helps further stabilize the vitamin A since retinol is easily oxidized. Antioxidants are also typically added to aid with vitamin A stabilization (Herdt and Stowe, 1991). Ingredients that attract water, such as salt and choline, can greatly reduce vitamin A activity because water will dissolve the gelatin matrix (Herdt and Stowe, 1991; Shurson et al., 2011).

One final point about vitamin A supplementation to cattle is that there has been evidence of ruminal degradation of preformed vitamin A supplements. Retinyl acetate was 67% degraded in vitro when incubated in rumen fluid from steers fed a 70% concentrate diet (Rode et al., 1990). A similar observation was made when retinyl acetate was incubated with rumen fluid from dairy cows fed either a 50% or 80% forage diet; ruminal degradation of the vitamin A supplement was 72% at 24 h in the 50% forage diet compared to 20% at 24 h for the 80% forage diet (Weiss et al., 1995). Thus, this should be taken into consideration when deciding on the amount of supplemental vitamin A to feed in the diet.

Conclusions

Vitamin A plays a role in a great deal of physiological processes throughout the body, resulting in a wide variety of ways a deficiency could have negative consequences on cow and calf health and performance. Status can be monitored with blood and liver samples, but liver is the more sensitive indicator of status as it appears to change with

dietary intake. Current suggested supplemental vitamin A levels (NASEM, 2016) have not been updated in over 50 years, and may not be appropriate for beef cows who are fed stored feedstuffs for an extended period of time. In cow/calf systems, the young beef calf seems to be most susceptible to vitamin A deficiency. Very few studies with cows have examined the impact of cow dietary intake of vitamin A on calf vitamin A status. The influence on calf status seems to be driven by vitamin A concentrations in the colostrum, which are the calf's primary vitamin A source at birth. More research is needed to better understand what supplemental vitamin A recommendations should be for beef cows on different diet types, and what supplemental amount of vitamin A cows should receive to ensure adequate vitamin A status of their calf.

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Table 1.1. Vitamin A content of feedstuffs commonly fed to beef cattle. Adapted from Pickworth et al. (2012).

Feedstuff	Vitamin A content (IU/kg of DM)^{1,2}
Hay, alfalfa	2913
Hay, fescue	2923
Silage, corn	6900
Corn gluten meal	3747
Corn, whole-shell	170
Corn, cracked	150
Corn, steam-flaked	137
Corn, high-moisture	359
Distillers grains w/ solubles, wet	800
Distillers grains w/ solubles, dry	482
Wheat straw	60

¹Expressed as vitamin A equivalents.

²Mean values reported only.

CHAPTER 2 - ASSESSMENT OF COW-CALF PERFORMANCE IN PERENNIAL FORAGE-BASED AND DRYLOT-BASED AUGUST-CALVING COW SYSTEMS

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Abstract

Conversion of perennial grasslands to cropland in U.S. Northern Plains has resulted in a need to evaluate alternative management strategies for cow-calf production. A 3-yr study was conducted to compare beef cow-calf performance in two different August-calving cow systems. Multiparous beef cows ($n = 228$; 5.7 ± 2.0 yr) were allocated to 8 herds, and herds were randomly assigned to a production system utilizing either perennial forage and corn residue grazing (PF) or a system that incorporated summer drylotting, fall grazing of a late-summer-planted cover crop, and corn residue grazing (DC). From mid-gestation (March/April) until pre-breeding (October), DC cows were limit-fed to meet requirements and PF cow grazed perennial pasture. Feed resource during the 47-d breeding period (November/December) varied by year and treatment. However, pregnancy rates did not differ ($P = 0.72$) between treatments within year. Both treatments went to corn residue at the time of weaning (late January) and grazed for 37 d. A treatment \times year \times time interaction ($P < 0.01$) was observed for cow BCS and calf BW.

In Year 1, cow BCS at pre-calving, pre-breeding, and end of the year was greater ($P < 0.01$) for PF (6.2 ± 0.06) compared to DC (5.3 ± 0.06). Weaning weights in Year 1 were greater ($P < 0.01$) for PF (186 ± 1.81 kg) than DC (175 ± 1.81 kg) calves. In Year 2, cow BCS was greater ($P < 0.01$) in PF (6.6 ± 0.06) than DC (6.1 ± 0.06) at pre-calving and pre-breeding, but did not differ (6.6 ± 0.06 ; $P = 0.09$) at the end of the year. At weaning in Year 2, calf BW of PF (201 ± 1.78 kg) was less ($P < 0.01$) than DC (212 ± 1.78 kg). Like Year 2, PF cows in Year 3 had a greater ($P < 0.01$) BCS at pre-calving compared to DC (7.7 ± 0.06 vs. 7.3 ± 0.06). However, pre-breeding BCS (6.8 ± 0.06) did not differ ($P = 0.98$). In Year 3, cow BCS at the end of the year was lower ($P < 0.01$) for PF (5.9 ± 0.06) than DC (7.1 ± 0.06) but calf BW at weaning did not differ (217 ± 1.81 kg; $P = 0.17$). Cow and calf performance were not sacrificed in August-calving cow-calf systems that utilized summer drylotting, fall cover crop grazing, and corn residue grazing.

Introduction

Between 2006 and 2011, almost 530,000 ha of grassland in the Northern Plains Region were converted to cropland used for corn and soybean production (Wright and Wimberly, 2013). In Nebraska, less pasture and increasing commodity prices have resulted in record-high land values (Jansen and Stokes, 2023), and consequently, have also increased pasture rental rates. The statewide average pasture cash rental rate in Nebraska has increased nearly 150% in the last decade, going from \$43/ha in 2012 to \$64/ha in 2022 (USDA-NASS, 2023). The reduction in pasture resources and increased pasture rental rates has prompted a need to evaluate alternative feeding and management strategies for cow-calf production. Previous work has demonstrated that pregnancy rates

are not negatively impacted and adequate cow body condition can be maintained when rations containing by-products such as distillers grains and low quality forages are fed to meet cow energy requirements (Shike et al., 2009; Anderson et al., 2013; Jenkins et al., 2015; Meter et al., 2018). Additionally, in areas where pasture acres are limited but grain crop production is abundant, corn residue is a readily available, inexpensive forage resource that can be used for fall/winter grazing (Klopfenstein et al., 1987). Combining corn residue grazing with summer confinement feeding of distillers grains and crop-residue-based diets has potential to be economically competitive with a perennial forage and corn residue grazing system when pasture prices are high (Warner et al., 2015).

Another forage option for fall grazing that has increased in popularity is cover crops planted in late summer following wheat or corn silage harvest. In addition to the soil health benefits and weed control cover crops provide (Adetunji et al., 2020), late summer-planted cool season species such as oats can be high-quality forage that maintains nutritive value through the fall and into winter, and can provide an opportunity for fall and early winter grazing (Drewnoski et al., 2018; Lenz et al., 2019). When this alternative fall forage option was incorporated into an August-calving cow system with summer confinement and corn residue grazing and compared to a traditional, spring-calving perennial pasture and corn residue grazing system, no differences in cow reproductive performance were observed (Carlson et al., 2022). In the same study, however, at the same day of age, calves in the summer confinement system had lighter weaning weights compared to calves in the spring-calving system. Given that the calves in the two systems were born at different times of year and experienced different weather events, it is unclear if the difference in weaning weights is due to management system or

time of year. Our null hypothesis was that cow and calf performance would not differ between the two August-calving cow systems. The objective of this study was to compare beef cow and calf performance in August-calving cow systems that utilized 1) perennial pasture and corn residue grazing or 2) summer drylot, fall cover crop grazing, and corn residue grazing.

Materials and Methods

Cattle

Research protocols were approved and monitored by the USDA, ARS, U.S. Meat Animal Research Center Institutional and Animal Care Committee in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (Ag. Guide, 2010).

Composite breed ($\frac{1}{4}$ Angus, $\frac{1}{4}$ Hereford, $\frac{1}{4}$ Simmental, $\frac{1}{4}$ Gelbvieh) cows bred to calve from late July through early September were used in the study. In the first year, bred cows ($n = 228$; 5.7 ± 2.0 yr) were stratified by birth year and randomly placed in 8 different herds. Four herds were then randomly assigned to a production system utilizing either perennial forage and corn residue grazing (PF) or a system that incorporated summer drylotting, fall grazing of a late-summer planted cover crop, and corn residue grazing (DC). In the two subsequent years, cows from a spring herd that were rolled over to the fall herd were added in February/March in manner to keep age stratification within the herds similar across the herds (Table 2.1). Throughout the study, cows remained in the herd they were assigned unless they were diagnosed to not be pregnant at palpation, if they or their calf were seriously ill or injured, or died before weaning.

General management of cattle

A timeline of the production year is available in Table 2.3. The study began in February. Calving each year began in August and lasted approximately 63 d. Cows in the PF and DC system calved while on pasture or in the drylot, respectively. In the event of a twin birth, one calf was removed from the dam at birth. Herds within PF and DC treatments were combined into 1 or 2 groups, respectively, during the breeding season in November. When calves were weaned (January/February), cows were sorted back into their herds and turned out on corn residue. Free choice mineral supplement was provided to cows while on corn residue. The new production year began in subsequent years when cows finished grazing corn residue (February/March) and were returned to either pasture or the drylot. Body condition scores (BCS; 1 to 9) were collected as whole scores on cows at the start of each production year (February), pre-calving (July), and pre-breeding (October). Pregnancy diagnosis via rectal palpation occurred in February. Weights on calves were collected at birth and weaning. Calves were transported to the onsite feedlot at weaning and penned by herd.

Cows were poured with insecticide in February at the time of palpation, pre-calving (July), and prior to breeding in October. All cows received Guardian (Merck Animal Health) and PregGuard Gold FP 10 (Zoetis) before calving and breeding, respectively. No estrous synchronization protocols were utilized on the cows for the breeding season. Calves were poured with insecticide and vaccinated with Pyramid 5 + Presponse (Boehringer Ingelheim) and Vision 7 with SPUR (Merck Animal Health) in

October, and received a second dose of Pyramid 5 + Presponse (all years) and Vision 7 with SPUR (Year 2 and 3 only) at the time of weaning.

In the winter and early spring months, cows in the PF system grazed either dormant warm-season dominated grass pastures or cool-season pastures. Warm-season grass pastures were grazed from May to late October each year. Predominant warm-season species included big bluestem (*Andropogon gerardii*), switchgrass (*Panicum virgatum*), yellow Indiangrass (*Sorghastrum nutans*), and little bluestem (*Schizachyrium scoparium*). Smooth bromegrass (*Bromus inermis*) was the predominant cool-season species in the cool-season pastures, but was also a minor component of the warm-season pastures. Kentucky bluegrass (*Poa pratensis*), sedges (*Carex spp.*), foxtails (*Setaria spp.*), and perennial forbs made up approximately 25% of the vegetation in the cool-season pastures.

Drylot cows were penned by herd and placed in pens that were 122 m × 61 m. Each pen had a shade structure and 46 m of bunk space. Each pen had a creep area to allow calves access to alfalfa-grass hay. The same pens were used all three years of the study for the drylot treatment. Pens were cleaned each year after cows were removed to graze cover crop.

General management year one (2019)

Cows were placed on study February 15. The calving season began on August 4 and concluded on September 26. Bulls were turned in on November 5 for 44 d, and the cow to bull ratio was 25:1. Calves were weaned on January 14 at 142 days of age (DOA), and cows were sorted back into their herds and subsequently turned out onto corn residue

for 31 d. Pregnancy diagnosis occurred on February 13 following corn residue grazing. Due to limited residue availability, cows were offered free-choice alfalfa/grass hay for the duration of the corn residue grazing period.

Perennial forage-based system year one

Cows were placed on dormant forage pastures and fed free-choice alfalfa/grass hay starting in February until the middle of April and herds were managed as separate treatment groups. Cows grazed pastures from mid-April until October 25, at which time all herds were then combined into a single group and moved to a stockpiled field of brown mid-rib forage sorghum for breeding. Calves were creep fed ad libitum alfalfa hay surrounded by a single-wire fence beginning on October 31. When bulls were turned in on November 5, cows were offered free-choice alfalfa/grass hay.

Drylot/cropland system year one

Cows were placed in the drylot beginning in February and fed a total mixed ration (TMR; Table 2.2) that consisted of corn stalks and wet distillers grains with solubles (WDGS). Additionally, cows received 0.23 kg/hd of a supplemental pellet that contained vitamins, minerals, and 450 mg Rumensin/kg dry matter. Starting two weeks before expected calving date, dry-rolled corn was added to the diet, and supplemental pellet amount was increased to 0.45 kg/hd.

Starting October 1 while cows were in the drylot, calves were creep fed ad libitum alfalfa hay. Cows were sorted into two breeding groups such that DC treatment groups were equally represented within each breeding group. Cover crop was planted late in this

year and was not ready for grazing by October 25, so one group was placed on an alfalfa/orchard grass mix pasture and the other group was placed on an alfalfa/endophyte-free tall fescue mix pasture.

General management year two (2020)

The production year started on February 14. Calving season started on July 25 and ended on September 26. Bulls were placed with cows on November 5 for 46 d, and cow to bull ratio was 15:1 (PF) or 20:1 (DC). Calves were weaned at 156 DOA on January 28, and cows were palpated on February 1 and sorted back into their herds before being turned out to corn residue for 43 d. Supplemental alfalfa/grass hay was provided free-choice to cows while grazing corn residue starting on February 9.

Perennial forage-based system year two

On February 14, cows were placed on dormant forage pastures until April, and grazed summer pasture until the end of October. Cows began receiving supplemental alfalfa/grass hay on September 8. Because of limited pasture availability due to drought, cows were maintained in their treatment groups and moved to the drylot on October 29 for breeding. Cows were fed a TMR that consisted of corn stalks, WDGS, and dry-rolled corn to meet energy requirements (Table 2.2). In addition, cows received 0.45 kg/hd/d of a supplemental pellet that supplied vitamins, minerals, and 450 mg Rumensin/kg DM. Starting on October 30, calves were allowed ad libitum access to alfalfa hay via a single-wire fence creep area.

Drylot/cropland system year two

From February 14 through October 27 rations and management of cows was as described in Year 1. Calves were creep fed alfalfa hay as described in Year 1 beginning September 29 until cattle were moved to cover crops. Cows were sorted into two breeding groups such that DC treatment groups were equally represented within each breeding group and placed on a cover crop. The cover crop was planted August 22-23 using 61.6 kg/ha Shelby 427 oats, 22.4 kg/ha cereal rye, and 3.4 kg/ha Trophy rapeseed. Breeding groups started grazing cover crop on October 28, and bulls were with cows from November 5 to December 21. Pairs continued to graze cover crop for an additional 31 d following the breeding season. Beginning January 21, cows were provided free-choice alfalfa hay until calves were weaned and cows were moved to corn residue. Cows were returned to the drylot after corn residue grazing ended on March 12.

General management year three (2021)

The final year of the study began in mid-March when the corn residue grazing ended from the previous year. Calving season went from August 2 to September 27, and calves were weaned at 163 DOA on February 4. Bulls were placed with cows for breeding on November 16 and the breeding season lasted for 49 d, and the cow to bull ratio was 11:1. The study ended after cows were palpated on February 9.

Perennial forage-based system year three

Cows were placed on pasture and received no additional forage supplementation until December 1 during breeding, at which time they began receiving free-choice

alfalfa/grass hay. On October 25, all herds in the PF treatment were combined into a single group for breeding and were moved to a single dormant forage pasture. Calves were ad libitum creep fed alfalfa hay as described in Years 1 and 2 starting January 1.

Drylot/cropland system year three

Cows received a TMR from mid-March to October that consisted of corn silage, ground alfalfa hay, and supplemental pellet (Table 2.2). The cover crop was planted August 27 using the same oat, rye, and rapeseed mix as described in Year 2. Beginning September 22 until cattle were moved to cover crops on November 2, calves were creep fed alfalfa hay as described in Years 1 and 2. Cows were divided into two breeding groups as described in Year 2. Bulls were with cows from November 16 to January 4, and pairs continued to graze cover crop for an additional 29 d following the breeding season.

Sample collection and analysis

Before herds were placed on dormant pasture, forage availability was sampled on each pasture. Forage samples were subsequently collected every two weeks to assess forage availability. When spring forage growth began, a 9.3-square meter exclusion area in each replicate pasture was created. Every two to three weeks, forage availability in the pasture and the exclusion area from the previous two weeks was sampled to determine forage removal. When cows were moved to a new pasture, an exclusion area was installed, forage available was measured. Before cows were placed on corn residue, forage availability was determined, and a 9.3-square meter exclusion area was created. When cattle were removed, available forage in the exclusion areas and the remaining in

the field were determined. Diets fed to cows in the drylot were sampled daily and a weekly composite was made. Forage and diet samples were dried at 60°C to determine dry matter content. Crude protein and TDN content of the drylot diets was calculated using book values (NASEM, 2016) and a TDN value of 108% was used for WDGS (Loy et al., 2008).

Performance calculations

Pregnancy rate, calving rate, and weaning rate were calculated with total number of cows exposed as the denominator. A cow was counted as exposed if she entered the breeding season and made it to palpation. Cows that were enrolled on study as replacements at the time of palpation following a breeding season were not included in cow exposed counts. All calves born full-term, including those that were stillborn, were included in the calving rate calculations. Twins were counted as one calf for calving and weaning rate calculations; however, if a twin made it to weaning, their weaning weight was not used in any statistical analyses. There was no difference ($P = 0.11$; data not shown) in the percentage of twins born between treatments. To estimate kilograms of calf weaned per cow exposed, an average weaning weight of the single-born calves in each herd was multiplied by the total number of calves weaned (including the twin born calves that made it to weaning) and divided by the total number of cows exposed.

Statistical analysis

For all analyses herd was considered the experimental unit. Cow BCS collected at pre-calving (July), and breeding (October), and palpation (February or March) were

designated as periods. Cow BCS was analyzed using the MIXED procedure of SAS (Cary, NC) as a repeated measure with the subject equal to cow within year. Fixed effects included treatment, year, period, treatment by year, treatment by period, year by period, and treatment by period by year with the denominator degrees of freedom set to the Kenward-Roger method. Cow nested within herd by treatment was random. The proportion of cows pregnant was analyzed in the GLIMMIX procedure of SAS (Cary, NC). The model included treatment, year, and treatment by year. Cow nested within herd by treatment was random, and the `_RESIDUAL_` keyword was included in the RANDOM statement. The data were analyzed as binomial with a logit link and an ARH(1) covariance structure, and denominator degrees of freedom were set to the Kenward-Roger method. The proportion of calves born and weaned were analyzed as binomial with a logit link in the GLIMMIX procedure of SAS (Cary, NC), and the model included treatment, year, and treatment by year as fixed effects. Kilograms of calf weaned per cow exposed were analyzed in the MIXED procedure of SAS with treatment, year, and their interaction as fixed effects.

Birth and weaning BW time points for calves were each considered a period. Twins were removed from the data set for statistical analysis. Calf BW was analyzed using the MIXED procedure of SAS (Cary, NC) as a repeated measure with the subject equal to calf nested within year. The covariance structure was set to compound symmetry. Fixed effects included treatment, year, period, sex, treatment by year, treatment by period, period by year, period by sex, and treatment by period by year with the denominator degrees of freedom set to the Kenward-Roger method. Calf within herd by treatment by year by sex was a random effect. Days of age at weaning was analyzed in

the MIXED procedure of SAS (Cary, NC). The model included fixed effects of treatment, year, and treatment by year with denominator degrees of freedom set to the Kenward-Roger method.

Results and Discussion

Cow performance

Pregnancy rates, calving rates, and weaning rates did not differ ($P \geq 0.57$; Table 2.4) between treatments within year. This agrees with Carlson et al. (2022), who also observed no differences in pregnancy, calving, or weaning rates between a spring-calving perennial forage cow-calf system and a summer-calving cow-calf system with confinement and cover crop grazing components. During breeding, cows in the spring-calving system would have been grazing smooth brome grass pastures, while summer-calving cows were grazing a late-summer-planted oat monoculture. Body condition going into the breeding season for both calving groups was adequate with majority of cows falling between BCS 5 to 7. No difference in pregnancy rates between perennial forage-based and confinement cow-calf systems have also been observed by Anderson et al. (2013) and Myerscough et al. (2022). Cows in these studies were spring-calving, and the studies began around the time of breeding. Diets of cows in confinement during breeding in these studies were fed to meet nutrient requirements and consisted of some combination of crop residues, byproducts, grass hay, corn silage, and corn grain. Cows in each system in the study of Myerscough et al. (2022) were assigned to treatments the day they were artificially inseminated (AI), exposed to bulls 11 d following AI, and did not differ in body condition (mean BCS = 6.65). Anderson et al. (2013) did not report body

condition of cows, but noted a 48 kg and 25 kg decrease in body weight in confinement and pasture cows, respectively, during the breeding season, suggesting cows likely had adequate energy stores before breeding if the weight lost during breeding had no impacts on pregnancy rates, especially in the confinement cow system. Body condition is an estimate of the cow's energy reserves, and BCS at the time of breeding is a major factor affecting pregnancy rates (Selk et al., 1988). Differences in diet, therefore, do not seem to influence pregnancy rates as long as cows are in adequate body condition.

There was a treatment \times time \times year interaction ($P < 0.01$) observed for cow BCS (Table 2.5). In Year 1, BCS was greater ($P < 0.01$; Table 2.5) at pre-calving (July), pre-breeding (October), and the end of the production year (February) for PF compared to DC cows; however, cows in the DC treatment never dropped below a BCS 5 and were still considered to be in adequate condition. Differences in BCS between treatments were expected in Year 1 because PF cows could easily gain body condition when they were on summer pasture and not lactating, whereas DC cows were fed to maintain a BCS of 5 while in the drylot. Body condition declined from pre-calving (July) to the end of production year (weaning) in PF cows from 7.01 to 5.44 (SEM = 0.06 and 0.055, respectively), which is similar observation made by Carlson et al. (2022) and Myerscough et al. (2022) with cows managed in spring-calving perennial forage systems. Cows on the DC treatment essentially maintained body condition score throughout the year, a trend similar to what others have observed from breeding to weaning when comparing drylot and perennial forage systems (Carlson et al., 2022; Myerscough et al., 2022).

In Year 2, cow BCS was greater ($P < 0.01$) for PF than DC cows at pre-calving (July) and pre-breeding (October) but was not different ($P = 0.09$) between the treatments at the end of the production year (March). Cows in the PF system maintained a BCS of about 6.6 ± 0.059 throughout the production year; this was likely due to them being fed in the drylot during breeding to meet energy requirements. Cows in the DC treatment in Year 2 never fell below a BCS 6. In fact, BCS for DC cows increased from pre-breeding to end of the year, suggesting cover crop quality was high enough to add body condition to cows that were lactating. Lenz et al. (2019) reported that late-summer-planted oats (17.9% CP; 79.0% IVOMD) and brassicas (26.4% CP; 88.9% IVOMD) have good feed value for cattle, as they are high in nutritive value in the fall and can maintain quality throughout the fall and winter months. Others have shown that lactating cows grazing an oat monoculture in the fall from breeding to weaning were able to maintain BCS, but BCS distribution at weaning was less uniform than at breeding (Carlson et al., 2022). Authors speculated that the spread in BCS distribution at weaning meant that for some cows, energy requirements were not being met during lactation, while with other cows the oats exceeded their energy requirements.

In Year 3, BCS was greater ($P < 0.01$) for PF (7.71 ± 0.058) at the pre-calving time point (July) but was lower ($P < 0.01$) than DC cows at the end of the production year (February; 5.89 vs. 7.13; SEM = 0.061). Cow BCS was not different (6.84 ± 0.061 ; $P = 0.98$) between treatments at pre-breeding in October. Carlson et al. (2022) mentioned that BCS distribution of cows managed in a perennial forage spring-calving cow-calf system shifted from 6.0 and 7.0 at breeding (July) and moved closer to a 5.0 at weaning (October), which was a phenomenon observed in Year 1 for PF cows. The cows in the

perennial forage system were likely utilizing fat stores to meet energy requirements (Carlson et al., 2022), as smooth bromegrass forage quality tends to decline over the summer grazing season, although this was not observed by Carlson et al. (2022). Instead, authors suggested that biomass availability was limited late in the grazing season. In contrast, cows on the PF treatment in the current experiment were grazing both cool- and warm-season grass pastures throughout the year. Warm-season grasses hit peak quality and production in the summer months, while cool-season grasses hit peak production and quality in early spring, with some regrowth in the fall. The benefit of having both cool- and warm-season grass pastures to graze allows for extending the grazing period, but also for meeting protein and energy needs for a larger part of the grazing season (NDSU Extension, 2020).

Calf performance

A treatment \times year interaction ($P < 0.01$) was observed for age at weaning (Table 2.6). In Year 1, calf age at weaning for PF calves was lower ($P < 0.01$) than DC calves. Age at weaning was not different ($P = 0.08$) between the two systems in Year 2, but PF calves were older ($P < 0.01$) at weaning than DC calves in Year 3. The goal was to target a similar age at weaning each year, and although the differences in age are statistically significant in Years 1 and 3, they were only 6 d apart in Year 1 and 4 d apart in Year 3. Each year, calves from each system were weaned within a day of each other, and bulls were turned out with cows in each system on the same day; therefore, age differences are likely caused by minor differences in day of conception and gestation length.

There was a significant treatment \times time \times year interaction ($P < 0.01$) for calf BW (Table 2.6). In Year 1, birth weights of calves were not different ($P = 0.36$; Table 2.6) between treatments, with average weight being 39.0 ± 1.77 kg. At weaning, however, BW of PF calves was 11 kg greater ($P < 0.01$) than DC calves (186 vs. 175 kg; SEM = 1.81 kg, respectively). Like Year 1, calf birth BW in Year 2 was not statistically different ($P = 0.26$) between PF (37.2 ± 1.71 kg) and DC (39.9 ± 1.71 kg) groups, but unlike Year 1, weaning weights were greater ($P < 0.01$) for calves in DC compared to PF by 11 kg. Body weight of calves at birth and weaning was not different ($P \geq 0.13$) between PF or DC in Year 3. The percentage of heifers weaned was not different ($P = 0.17$; data not shown) between treatments within year, so this was unlikely the cause of different responses in weaning weights between the two systems across years.

The lack of difference in birth weights between treatments has been observed in other studies with beef cows evaluating perennial forage-based and confinement feeding systems (Perry et al., 1974; Anderson et al., 2013; Burson, 2017; Carlson et al., 2022). In the current study, alfalfa hay was provided in a fenced in creep area to calves ad libitum in both PF and DC treatments about a month before breeding, but PF calves also had access to summer pasture in addition to receiving the alfalfa hay, whereas DC calves would have limited access to the cow TMR as it was consumed relatively quickly (Table 2.3). Forage type, amount, and quality could have led to differences in weaning BW between the two treatment groups. Like Year 1 of this study, weaning BW of calves reared in perennial forage-based systems has been observed to be greater compared to confinement systems (Burson, 2017; Carlson et al., 2022). Calves in the pasture system in the study of Burson et al. (2017) did not receive any creep feed while on volunteer wheat

pasture in the spring, whereas calves in confinement received a creep diet containing 16.8% CP and 0.98 Mcal NEg/kg DM. Dams of confinement calves received a diet of wet corn gluten feed, Old World bluestem hay, ground cotton burrs, and cracked corn, which calves would have had access to as well. However, the pasture supplement provided to the calves' dams for the duration of the study contained 21.8% CP and 1.28 Mcal NEg/kg DM, and was fed at a rate of 4.1 kg DM/hd/d. It is possible that pasture calves in this study consumed some pasture supplement in addition to dormant forage, which may have caused the 0.12 kg/d increase in their ADG over calves in confinement from birth to weaning and increased BW at weaning. Calf weaning weights at 168 d of age were also observed to be greater in a spring-calving pasture-based cow-calf system compared to a semi-confined summer-calving cow-calf system (Carlson et al., 2022). Authors attributed this observation to differences in environmental conditions the calves were facing the time of year they were born. Calves in the summer-calving system would have been experiencing colder temperatures from birth to weaning, which could have resulted in a lower BW at weaning. Neither group of calves were provided creep feed prior to weaning.

Weaning weights of PF calves in Year 2 were lower compared to DC calves. The greater weaning weight observed in DC calves in Year 2 may be attributed to the feed resource available to pairs. Prior to breeding, pairs in the DC treatment were in the drylot and PF pairs were on pasture (Table 2.3). Calves during this time frame in the DC group would maybe have had access to the cow's limit-fed TMR, and they also received ad libitum access to alfalfa hay beginning September 29. Because Year 2 (2020) was a drought year, forage quality and quantity in the pastures grazed by PF pairs was likely

less than Year 1. From pre-breeding (October) until weaning, DC pairs were grazing an oat-dominated cover crop mix and pairs in the PF treatment received a TMR in the drylot. Late-summer-planted oats and brassicas are quite high in CP content and digestibility in the fall, and they tend to maintain this quality throughout the fall and winter months (Cox-O'Neill et al., 2017; Lenz et al., 2019). When PF pairs were in the drylot, they were limit-fed and it is likely that calves did not consume much of the TMR provided; thus, most of their forage intake was likely coming from the alfalfa hay they were creep-fed. Again, it is possible the different quantity and quality of feedstuffs pairs had access to in each system, especially from pre-breeding to weaning, impacted calf performance in Year 2. Myerscough et al. (2022) reported that calves raised in a confinement system from approximately 84 d of age weighed 31 kg more at weaning than calves of a similar age in a pasture system. In that study, calves in confinement were receiving ad libitum access to a TMR identical to the one the cows were receiving, while calves in the pasture system were only creep fed for the final 3 wk of the pre-weaning period. Milk production was also greater for drylot cows compared to pasture cows, but milk nutrient composition was not different. This study would suggest that feed availability, intake, and nutrient content before weaning could have an impact on calf weaning weight.

Year 3 calf weaning weights were not different between PF and DC systems. Similarly, Perry et al. (1974) observed no difference in BW at weaning between calves in confinement and forage-based spring-calving cow-calf systems. One forage system was grazing of Kentucky bluegrass from May to October, corn residue from November to January, and hay from February to April. The other forage system was grazing of Kentucky bluegrass pastures from May to June and October to November, sorghum-

sudan from July to September, and corn stover silage from December through April. Cows fed in the drylot received only corn silage in an amount to meet nutrient requirements; thus, calves in the confinement system in that study only had access to the corn silage their dams received, and had to compete with cows for feed since no creep feed was provided. In the current study, DC calves were creep fed alfalfa hay while in the drylot with their mothers, and also had the opportunity to graze high-quality cover crop from late October until weaning (Table 2.3).

Kilograms weaned per cow exposed was not different ($P = 0.94$) between treatments within year. There was 8% more kilograms of calf weaned per cow exposed for the DC system compared to the PF system in Year 2 (137 vs. 148 kg; SEM = 6.45 kg, respectively); however, this difference was not statistically significant ($P = 0.25$; Table 2.6). Kilograms of calf weaned per cow exposed in Year 3 was about 7% higher in the DC system than in the PF system (146 vs. 156 kg; SEM = 6.45 kg, respectively), but again, this difference was not statistically significant ($P = 0.29$; Table 2.6). In contrast, Carlson et al. (2022) reported a significant difference ($P < 0.01$) in kilograms weaned per cow exposed between two cow-calf systems; 49 kg less BW was weaned per cow exposed in a summer-calving partial confinement cow-calf system than in a spring-calving perennial forage system. This was the result of calves in the perennial forage system weighing 45 kg more at weaning. Even though differences in weaning weights varied between systems across years, no significant differences in kilograms of calf weaned per cow exposed between the PF and DC systems would mean no significant difference in total kilograms of calf available for sale at the time of weaning between the two systems. Therefore, revenue from calf sales at weaning may not be significantly

affected if August-born calves are managed in a system with summer drylot, fall cover crop grazing, and corn residue grazing.

Conclusion

Cow and calf performance were not sacrificed in the cow-calf system that combined summer drylotting, fall cover crop grazing, and corn residue grazing. Although differences in cow BCS were sometimes observed between the two production systems across years, all cows maintained adequate body condition throughout the study and no differences were observed in pregnancy rates. Based on these performance data, an August-calving cow-calf production system combining summer drylotting, fall cover crop grazing, and corn residue grazing could be a viable alternative when perennial forage is limited but ample cropland is available. However, viability of this system will ultimately depend on costs, which will vary between operations.

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Table 2.1. Cows on study at the start of each production year¹ by system. Each production year started in February. Systems consisted of perennial pasture and corn residue grazing (PF) or summer drylot, fall cover crop grazing, and corn residue grazing (DC).

Herd ID	DC				PF			
	1	2	3	4	5	6	7	8
Year 1 ¹	28	29	28	28	30	28	29	28
Year 2 ²	29	30	30	29	31	31	30	29
Year 3 ³	35	36	37	33	40	39	39	34

¹ Year 1 (2019), Year 2 (2020), Year 3 (2021).

Table 2.2. Dietary and nutrient composition of rations fed to cows in the drylot.

Ingredient, % of DM	Year 1 and 2		Year 3	
	Gestation ¹	Lactation ²	Gestation ³	Lactation ⁴
Corn stalks	75.2	60.4	-	-
WDGS ⁵	22.5	22.4	-	-
Corn silage	-	-	48.4	48.3
Alfalfa hay	-	-	48.4	48.3
Corn, dry-rolled	-	14.6	-	-
Supplemental pellet ⁶	2.3	2.6	3.2	3.4
Diet nutrient content, % of DM				
CP	11.5	11.9	14.0	14.0
TDN	63.3	69.0	61.5	61.5

¹Fed at a rate of 11.7 kg DM/cow/d from February to two weeks prior to the start of calving to cows on DC treatment.

²Fed at a rate of 12.7 kg DM/cow/d from two weeks prior to calving until late October to DC cows; cows on PF treatment fed from late October to late January in Year 2.

³Fed at a rate of 9.14 kg DM/cow/d from February to two weeks prior to the start of calving to cows on DC treatment.

⁴Fed at a rate of 9.47 kg DM/cow/d from two weeks prior to calving until November 1 to DC cows.

⁵Wet distillers grains with solubles.

⁶Pellet provided vitamins, minerals, and supplied 103 and 205 mg Rumensin/cow/d when fed in gestation and lactation rations, respectively.

Table 2.3. Annual timeline of production stage and feed resource for two August-calving cow production systems. Systems consisted of perennial pasture and corn residue grazing (PF) or summer drylot, fall cover crop grazing, and corn residue grazing (DC).

Month	Study time point	Production stage	System	
			PF	DC
February	Palpation	Mid-gestation	Dormant pasture + supp. alfalfa/grass hay	Limit-fed in drylot
April		Mid-gestation	Pasture	
July	Pre-calving	Late gestation		
August	Calving	Early lactation		
October		Early lactation	Yr 1: forage sorghum	Yr 1: alfalfa/grass mix Yr 2 & 3: cover crop mix
November	Breeding	Late lactation	Yr 2: drylot Yr 3: dormant forage	
January	Weaning	Early gestation	Corn residue	Corn residue

Table 2.4. Effect of August-calving cow-calf system on cow reproductive metrics by year. Systems were 1) perennial pasture and corn residue grazing (PF) or 2) summer drylot, fall cover crop grazing, and corn residue grazing (DC).

Item	Treatment		SEM ¹	P-value ²
	PF	DC		
Pregnancy rate, %				
Year 1 ³	81.5	77.2	4.28	0.48
Year 2 ⁴	96.4	95.9	2.31	0.87
Year 3 ⁵	92.4	85.0	3.54	0.16
Calving rate ⁶ , %				
Year 2 ³	73.5	78.0	4.30	0.47
Year 3 ⁴	73.3	82.2	3.78	0.13
Weaning rate ⁷ , %				
Year 2 ³	68.4	70.0	4.64	0.81
Year 3 ⁴	67.5	72.0	4.20	0.46

¹Average SEM across treatments within each year.

²P-value for main effect of treatment within year shown. Treatment by year interaction was not significant ($P \geq 0.57$) for pregnancy rate, calving rate, or weaning rate. Main effect of treatment not significant ($P \geq 0.12$) for pregnancy rate, calving rate, or weaning rate.

³2019 breeding season.

⁴2020 breeding season.

⁵2021 breeding season.

⁶Number of full-term calves born divided by number of cows exposed. Twins were counted as a single calf.

⁷Number of calves weaned divided by number of cows exposed. Twins were counted as a single calf.

Table 2.5. Effect of August-calving cow-calf system on cow body condition by year. Systems were 1) perennial pasture and corn residue grazing (PF) or 2) summer drylot, fall cover crop grazing, and corn residue grazing (DC).

Item	Treatment		SEM ¹	P-value ²
	PF	DC		
Year 1				
Cow BCS ³				
Pre-calving (July) ⁴	7.01	5.42	0.060	<0.01
Pre-breeding (October) ⁵	6.27	5.42	0.061	<0.01
End (February) ⁶	5.44	5.08	0.055	<0.01
Year 2				
Cow BCS ³				
Pre-calving (July) ⁴	6.67	6.04	0.059	<0.01
Pre-breeding (October) ⁵	6.62	6.09	0.060	<0.01
End (March) ⁶	6.52	6.65	0.057	0.09
Year 3				
Cow BCS ³				
Pre-calving (July) ⁴	7.71	7.26	0.058	<0.01
Pre-breeding (October) ⁵	6.84	6.84	0.061	0.98
End (February) ⁶	5.89	7.13	0.061	<0.01

¹Average SEM across treatments within each time point.

²P-value for main effect of treatment within time point. 3-way interaction between treatment, time point, and year was significant ($P < 0.01$) for cow BCS.

³Body condition score (1 = emaciated to 9 = obese).

⁴PF cows grazing perennial forage, DC cows limit-fed in drylot to meet energy requirements.

⁵Body condition prior to bull turn-out for breeding. In Year 1, PF cows placed on stockpiled forage sorghum and DC cows placed on alfalfa/grass pivots. In Year 2, PF cows placed in the drylot and DC cows placed on cover crop. In Year 3, PF cows placed on dormant perennial grass pastures and DC cows placed on cover crop. Breeding season was 44, 46 and 49 d in Years 1 through 3, respectively.

⁶Body condition score following corn residue grazing.

Table 2.6. Effect of August-calving cow-calf system on calf performance. Systems were 1) perennial pasture and corn residue grazing (PF) or 2) summer drylot, fall cover crop grazing, and corn residue grazing (DC).

Item	Treatment		SEM ¹	<i>P</i> -value ²
	PF	DC		
Year 1 (2019)				
Calf BW ³ , kg				
Birth	37.9	40.2	1.77	0.36
Weaning (January) ⁴	186	175	1.81	<0.01
Age at weaning, d	139	145	0.44	<0.01
kg weaned/cow exposed ⁵	-	-	-	-
Year 2 (2020)				
Calf BW ³ , kg				
Birth	37.2	39.9	1.71	0.26
Weaning (January) ⁴	201	212	1.78	<0.01
Age at weaning, d	155	156	0.46	0.08
kg weaned/cow exposed ⁶	137	148	6.45	0.25
Year 3 (2021)				
Calf BW ³ , kg				
Birth	40.7	37.0	1.75	0.13
Weaning (February) ⁴	215	219	1.81	0.17
Age at weaning, d	165	161	0.47	<0.01
kg weaned/cow exposed ⁶	146	156	6.45	0.29

¹Average SEM across treatments within each time point.

²*P*-value for main effect of treatment within time point. 3-way interaction between treatment, time point, and year was significant ($P < 0.01$) for calf BW. Treatment by year interaction was significant ($P < 0.01$) for age at weaning, and not significant ($P = 0.94$) for kg weaned/cow exposed. Main effect of treatment for kg weaned/cow exposed not significant ($P = 0.13$).

³Excludes twins.

⁴Calves weaned prior to cow turnout on corn residue.

⁵Not calculated in Year 1 because all cows were bred prior to enrollment on study.

⁶Average weaning weight of single-born calves in each herd was multiplied by the total number of calves weaned (singles plus twins counted as a single) and divided by the total number of cows exposed.

CHAPTER 3 - EVALUATING RELATIONSHIPS BETWEEN PLASMA AND LIVER RETINOL CONCENTRATIONS IN THE BEEF COW AND HER CALF

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Abstract

In the young calf, vitamin A is particularly important for immune system maturation, and calves rely on colostrum at birth to supply vitamin A. Cow vitamin A status may influence colostrum vitamin A concentrations, which can impact vitamin A status of their calves. Very little is understood about this relationship between beef cow and calf vitamin A status. Furthermore, limited data is available on how plasma and liver retinol concentrations in cattle are related to one another. The objective of this study was to examine relationships between liver and plasma retinol concentrations in the cow, calf, and within cow-calf pairs. Multiparous MARC II beef cows ($n = 120$; 6.4 ± 1.2 years of age; 592 ± 58 kg BW) in mid-gestation were provided supplemental vitamin A as retinyl acetate in a supplemental pellet at a rate of either $9,643 \pm 460$ IU/d ($n = 30$) or $25,044 \pm 2,072$ IU/d vitamin A ($n = 90$; current NASEM recommendation = 33,000 IU/d). Cows were individually fed using Calan gates a diet consisting of alfalfa hay, corn silage, and supplemental pellet for 144 d (111 d pre-calving and 32 d post-calving). Basal diet

vitamin A concentration was 490 IU/kg of DM. Mean initial liver concentration of cows was 830 μg retinol/g of DM. To assess vitamin A status, liver biopsies and plasma samples were collected on cows and calves at the end of the supplemental period when calves averaged 32 ± 7 days of age (DOA). Pearson correlations were used to test for linear relationships between cow liver and plasma retinol concentrations, calf liver and plasma retinol concentrations, and liver and plasma retinol concentrations between the cow and her calf. No linear relationship ($P = 0.11$; $r = 0.15$) was observed between liver and plasma retinol in cows. In the present study, mean cow liver retinol concentration (482 ± 182 SD $\mu\text{g}/\text{g}$ of DM) fell within the current adequacy reference range of 300–700 $\mu\text{g}/\text{g}$ of DM. Cow plasma retinol (272 ± 40 SD ng/mL) was slightly below the reference range of 300–800 ng/mL. A positive correlation ($P < 0.01$; $r = 0.37$) was detected between calf liver (51 ± 27 SD $\mu\text{g}/\text{g}$ of DM) and plasma (190 ± 47 ng/mL) retinol concentrations. Both were below what would be considered adequate (100–350 $\mu\text{g}/\text{g}$ of DM in liver; 225–325 ng/mL in plasma) for calves at 32 DOA. There was a positive correlation ($P < 0.01$; $r = 0.33$) between cow and calf liver retinol, suggesting that as cow retinol liver concentrations increased, calf liver retinol concentrations increased. It appears that despite cows having adequate liver retinol concentrations when less than the current NASEM recommendation for vitamin A was fed, it did not result in calf liver retinol stores that would be considered adequate given current reference ranges.

Introduction

Vitamin A is well-known for its role in vision, but it is also important for proper immune function and epithelial integrity, specifically in the gastrointestinal and

respiratory tracts (Semba, 1998). Clinical deficiency is rare, but marginal deficiencies can still impact cow productivity and potentially calf health. Calves are born with very low vitamin A stores because placental transfer of vitamin A is quite low (Malone, 1975), and their primary source at birth is colostrum. Vitamin A concentrations in colostrum have been reported to be six (Schweigert, 1989) to fourteen (Branstetter et al., 1973) times greater than that of milk, so colostrum is critical for establishing vitamin A stores in the young calf. Because of the role that vitamin A plays in maintaining epithelial integrity in the gastrointestinal and respiratory tracts, calves that do not get enough vitamin A from colostrum may be at increased risk for diarrhea and respiratory disease (Stewart and McCallum, 1938).

Vitamin A status can be evaluated using liver or plasma samples. While plasma is the easier sample to obtain, it is not a very sensitive indicator of vitamin A status. Plasma concentrations in general do not fluctuate (Blomhoff et al., 1990; Goodman, 1984) unless liver stores become severely depleted (Dowling and Wald, 1958; Olson, 1984) or when liver storage capacity is exceeded (Olson, 1984). Timing of sampling can influence plasma vitamin levels as well, and perceived vitamin A status based on plasma can change with stage of production. Plasma concentrations of vitamin A in dairy cows have been noted to decrease from late gestation to a nadir at parturition, then begin to gradually increase in the early weeks of lactation (Goff and Stabel, 1990; Oldham et al., 1991). This occurs in part because of colostrogenesis (Goff and Stabel, 1990; Goff et al., 2002). Oldham et al. (1991) observed a decrease in serum vitamin A concentrations in dairy cows about 4 months prior to calving, regardless of level of supplemental vitamin A

included in the diet. This study suggests that plasma samples would not fluctuate with vitamin A intake, and therefore may not be a good indicator of status.

Liver samples are more reflective of changes in dietary vitamin A intake, as they increase when vitamin A intake exceeds physiological need, or decrease when vitamin A intake is insufficient to meet the body's needs (Olson, 1984). Approximately 90% of total body vitamin A is stored in the liver (Olson, 1984; McDowell, 2000), and liver vitamin A is used to maintain plasma retinol concentrations. For example, in finishing steers fed a carotene-free diet supplemented with 0, 55, 220, 440, or 1100 IU/kg BW, Frey et al. (1947) observed no relationship between serum and liver concentrations of vitamin A at time of slaughter, as serum concentrations plasma retinol concentrations did not differ significantly between the 3 higher levels of supplementation (Frey et al., 1947). Hepatic vitamin A concentrations increased up to 1100 IU/kg BW of supplemental vitamin A, suggesting a strong relationship between liver concentrations and dietary vitamin A intake. Thus, liver samples are more useful for determining the total body reserves of an animal, whereas blood samples help determine if a severe deficiency or toxicity is present.

There are very few studies that illustrate the relationship between liver and plasma retinol concentrations in the young calf. In general, they have concluded that plasma vitamin A concentrations are poor predictors of liver retinol concentrations, especially as liver concentrations increased (Hibbs and Kraus, 1947; Thomas and Moore, 1952; Wheeler et al., 1957). Of these few studies, only one has evaluated this relationship in dairy calves (Wheeler et al., 1957), while the rest have been with dairy calves (Hibbs and Kraus, 1947; Thomas and Moore, 1952). Furthermore, there is no data available on how

beef cow liver and plasma retinol concentrations correlate to plasma and liver retinol concentrations in the beef calf. Dairy calves are managed very differently compared to beef cows; dairy calves are removed from the dam at birth and fed milk replacer, and beef calves remain with their dams and will consume the colostrum produced by their dam. Understanding this relationship would be valuable because cow vitamin A status may provide a way to predict calf vitamin A status without having to collect liver and plasma samples on calves. The objective of this study was to identify the relationship between cow and calf vitamin A status using plasma and liver samples. Our hypothesis was that calves would have adequate liver vitamin A concentrations as long as their dams had liver vitamin A concentrations that fell within adequate reference ranges.

Materials and Methods

Cattle and feeding

Research protocols were approved and monitored by the USDA, ARS, U.S. Meat Animal Research Center Institutional and Animal Care Committee in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

Composite breed ($\frac{1}{4}$ Angus, $\frac{1}{4}$ Hereford, $\frac{1}{4}$ Simmental, $\frac{1}{4}$ Gelbvieh) cows in mid-gestation (6.4 ± 1.2 years of age; $n = 120$) who had previously been grazing on pasture were utilized in this study. Initial BW of cows at the start of the study was 592 ± 58 kg. Cows received one of two different vitamin A supplementation levels: $9,643 \pm 460$ IU/d ($n = 30$) or $25,044 \pm 2,072$ IU/d vitamin A ($n = 90$). These levels were approximately one-third and two-thirds of the current NASEM (2016) recommendation of 2,800 IU/kg

DM for gestating beef cows weighing 591 kg consuming 2.0% of BW in DM per day (33,000 IU/d). They were individually fed using a Calan Broadbent Feeding system (Northwood, NH, USA) from 111 days pre-calving to 32 days post-calving. Cows were trained for 5 weeks prior to the start of the study to use the Calan gates. The ration consisted of a fixed amount of pelleted supplement that provided vitamin A in the form of retinyl acetate (Zhejiang NHU Company, Ltd.; China) and the remainder consisted of 77% ground alfalfa/grass hay and 19% corn silage on a dry matter basis. Mean vitamin A intake from the basal diet was $4,583 \pm 649$ IU/d, which was estimated from the calculated basal dietary vitamin A concentration of 490 IU/kg. Basal dietary vitamin A was calculated assuming 1000 μg of β -carotene is equal to 400 IU of vitamin A (McDowell, 2000). Cows were fed to meet a ME maintenance requirement of 138 kcal ME/ BW $\text{kg}^{0.75}$. The amount of hay and corn silage was increased as pregnancy progressed to meet increased energy requirement of the conceptus (NASEM, 2016). Metabolic body size at BCS 5.5 was calculated as the cow's BW plus 45 kg for every BCS less than 5.5 or minus 45 kg for every BCS over 5.5, then raised to the 0.75 power (NRC, 1996), and subsequent feed intakes were calculated from this weight. Cows were fed once daily in the morning and orts were determined every 7 d.

Sample collection and analyses

Diet samples were collected daily and composited by week, and were stored at -20°C until time of analysis. Diet samples were dried at 60°C for 72 h in a forced-air oven to determine dry matter content. To determine basal dietary vitamin A concentration, one diet sample from each supplemental vitamin A level at the end of the study was sent to a

diagnostic lab (Michigan State University Veterinary Diagnostic Lab; Lansing, MI) for β -carotene analysis using ultra performance liquid chromatography (AOAC, 2011; method 2001.13).

On d 0 (111 d pre-calving), cows were weighed and body condition scored. A blood sample (20 mL) and a liver biopsy were also collected at this time. Blood samples and liver biopsies were collected from cows again on d 144 (32 d post-calving), and from calves at 32 ± 7 d of age. All blood samples were collected via jugular venipuncture into EDTA tubes (BD Vacutainer; Becton, Dickinson, and Company, Franklin Lakes, NJ) and stored on ice for transport to the lab. Liver biopsies were collected using the method of Engle and Spears (2000) for determination of liver vitamin A. Blood was centrifuged and supernatant was placed in 2-mL microcentrifuge tubes and frozen at -80°C until analyzed for vitamin A. Liver samples were rinsed with PBS and collected into 2-mL microcentrifuge tubes, frozen in liquid N, and stored at -80°C until time of vitamin A analysis.

Liver samples were homogenized via pulverization using a cryogenic grinder (Freezer/Mill 6870D; SPEX Sample Prep, Metuchen, NJ) prior to vitamin A analysis. On day of analysis, liver samples were retrieved from storage and immediately placed in liquid N. Tubes were then removed from liquid N and broken to remove tissue. Tissue was quickly placed in a weigh boat and placed on dry ice until transfer into cryogenic grinding vials resting in liquid N. A pre-cooled stainless steel rod was placed in each vial, and vials were then capped with a room-temperature stainless steel plug before placement into the cryogenic grinder. Once ground, liver samples were transferred from the vials into aluminum weigh boats sitting on dry ice. Samples were then transferred into 5-ml

microcentrifuge tubes and stored on dry ice until weighing. Pulverized samples were weighed into 50-mL falcon tubes and placed on dry ice.

Vitamin A concentrations in liver and plasma samples were determined utilizing a portable fluorometer (iCheck Fluoro™, BioAnalyt GmbH, Teltow, Germany). Extraction vials designed for use with the fluorometer contain 98% hexane, 1% isopropanol, and 1% ethanol. Distilled water was added to liver samples to achieve a dilution factor (DF) of approximately 100 or 50 for cow and calf liver samples, respectively. Falcon tubes were then capped and shaken by hand for 1 min. Immediately afterwards, 500 µL of solution was injected into extraction vials and shaken intensively for 10 s. Extraction vials were then centrifuged at 650 rpm for 10 min to achieve phase separation. Vials were inserted into the fluorometer and vitamin A concentration was measured. A pig liver was obtained and multiple subsamples were collected to test this extraction procedure prior to sample analysis. Accuracy of the procedure was verified by sending subsamples from this liver to a commercial laboratory (Michigan State University Veterinary Diagnostic Lab; Lansing, MI) where they were analyzed for vitamin A concentration via ultra performance liquid chromatography using modified procedures from Schmitz et al. (1991) and Rettenmaier and Schuep (1992). All readings were expressed as µg retinol equivalents (RE)/L liver. Once analyzed for vitamin A content, the remainder of the liver sample from each animal at each time point was dried for 24 h at 100 °C in a forced-air oven to determine DM. Vitamin A concentration of liver is reported as µg retinol/g DM.

Sample preparation of plasma was done as described by the iCheck Fluoro™ manufacturer. Briefly, plasma was thawed and 500 µL of plasma was injected into extraction vials and shaken intensively for 10 s. Vials were allowed to settle until phase

separation was complete (~ 5 min). Vials were inserted into the fluorometer and vitamin A concentration was measured. All readings were expressed as μg retinol equivalents (RE)/L plasma.

Statistical Analysis

Pearson correlations (CORR procedure of SAS; Cary, NC) were used to test for linear relationships between cow liver and plasma retinol concentrations, calf liver and plasma retinol concentrations, and liver retinol concentrations between the cow and her calf. Significance was declared at $P \leq 0.05$.

Results and Discussion

Because cows had recently spent time on green grass, initial liver retinol concentrations (mean 830 ± 288 SD $\mu\text{g/g}$ DM) of cows were well above adequate. By 32 days post-calving, mean cow liver retinol concentration (482 ± 182 SD $\mu\text{g/g}$ DM) had decreased but was still considered adequate based on the current reference range of 300–700 $\mu\text{g/g}$ DM (Puls, 1994). Cow plasma retinol (272 ± 40 SD ng/mL) was slightly below the reference range of 300–800 ng/mL (Puls, 1994). The decrease in liver retinol concentration indicates that dietary vitamin A (basal + supplemental) was not sufficient to maintain liver retinol concentrations, and cows had to utilize liver stores throughout gestation to maintain adequate plasma retinol concentrations. No linear relationship ($P = 0.11$; $r = 0.15$; Fig. 3.1) was observed between liver and plasma retinol in cows, which is not surprising because plasma retinol concentrations are tightly regulated and will not

fluctuate unless liver vitamin A concentrations are very low or exceed liver storage capacity (Olson, 1984).

A moderate positive correlation ($P < 0.01$; $r = 0.37$; Fig. 3.2) was detected between calf liver (51 ± 27 SD $\mu\text{g/g DM}$) and plasma (190 ± 47 SD ng/mL) retinol concentrations. Both were below what would be considered adequate (100–350 $\mu\text{g/g DM}$ in liver; 225–325 ng/mL in plasma; Puls, 1994) for calves at 32 days of age. A similar observation was made by Hibbs and Kraus (1947), who reported that plasma vitamin A concentrations were a good indicator of liver vitamin A at lower liver storage levels. It is suspected a correlation was observed here because most calves had liver retinol concentrations less than 100 $\mu\text{g/g DM}$ (Fig. 3.2), which may have been too low to allow the calves to maintain adequate plasma retinol concentrations. Plasma retinol concentrations will decrease when liver vitamin A concentrations become very low (Olson, 1984), and while mean plasma retinol concentrations of calves in the current study were not extremely below the adequate reference range, it may be indicative that 51 $\mu\text{g/g DM}$ is the point at which calf liver vitamin A stores may not be able to sustain adequate retinol concentrations in plasma. No illness or symptoms of vitamin A deficiency were observed in any calves of the current study despite having liver and plasma retinol concentrations below adequate reference ranges; however, other literature has reported minimal disease incidence in calves with liver and plasma vitamin A concentrations below the currently defined adequate reference ranges. Thomas and Moore (1952) indicated that at liver vitamin A concentrations in calves 4 to 6 mo of age less than 2.1 $\mu\text{g/g}$ on a wet basis (7 $\mu\text{g/g DM}$; assuming samples were 30% DM), plasma vitamin A concentrations were decreased. Plasma vitamin A concentrations at this liver

level were 114 ng/mL. Calves with liver vitamin A concentrations this low could be considered clinically deficient (Puls, 1994); however, authors did not report on any health parameters of these calves. The calves in the current study were approximately 1 mo of age when vitamin A status was assessed, but like Thomas and Moore (1952) observed, plasma retinol concentrations were much higher compared to liver retinol concentrations. Another study conducted with dairy calves observed only 5 cases of mild diarrhea over a 28-d period with liver vitamin A levels at 28 d of age ranging from 60 to 80 $\mu\text{g/g}$ DM, and plasma vitamin A concentrations of 150 ng/mL (Nezvesky et al., 1950). Swanson et al. (2000) observed less incidence of diarrhea and hyperthermic rectal temperatures in dairy calves from d 4 to 28 of age when supplemental vitamin A level increased from 2,300 IU to 44,000 IU/kg DM. When concentrations of less than 9,000 IU/kg DM were provided, decreases in liver and plasma retinol concentrations were observed, while calves receiving 9,000 IU/kg DM had essentially maintained liver and plasma retinol concentrations at 75 $\mu\text{g/g}$ DM and 94 ng/mL, respectively, through d 28. In contrast, we observed a much wider spread between liver and plasma retinol concentrations in calves at 30 d of age (51 $\mu\text{g/g}$ DM and 190 ng/mL, respectively). These studies, along with results from the current study, suggest that perhaps the threshold before seeing adverse health effects in young calves may be lower than retinol concentrations of 100 $\mu\text{g/g}$ DM and 225 ng/mL in liver and plasma, respectively.

There was a moderate positive correlation ($P < 0.01$; $r = 0.33$) between cow and calf liver retinol (Fig. 3.3), suggesting that as cow retinol liver concentrations increased, calf liver retinol concentrations increased. However, it appears that despite cows having adequate liver retinol concentrations, when supplemental vitamin A levels below current

recommendations were fed, it did not result in calf liver retinol stores that allowed for homeostatic regulation of plasma vitamin A. This is likely because cow liver retinol stores are not the only contributor to vitamin A in colostrum. Dietary vitamin A levels the cow receives during late gestation, as well as her liver vitamin A stores, affect the amount of vitamin A her calf receives to build its own liver vitamin A stores. Research in beef cattle indicates cow stores only contribute about 40% of the vitamin A found in colostrum, while the other 60% comes from the cow's diet (Branstetter et al., 1973; Tomlinson et al., 1974). Cows used in the research to figure out these contributions were supplemented 45,000 IU/d (Branstetter et al., 1973) or 50,000 IU/d (Tomlinson et al., 1974), in diets consisting of primarily alfalfa-grass hay supplemented with corn, so it is unclear if these contributions from cow liver vitamin A stores and cow dietary vitamin A would be similar in the current study where cows were receiving less than 30,000 IU/d of supplemental vitamin A. Liver vitamin A concentrations in these studies were reported to be maintained at a minimum of 200 µg/g (unclear if on a wet or dry basis). Regardless, both of these studies demonstrate the importance of dietary vitamin A contributions to vitamin A in colostrum. More research is needed to understand contributions of dietary vitamin A intake and liver retinol concentrations to colostrum when vitamin A intake is not sufficient to build liver vitamin A stores in the calf.

Conclusion

Colostrum is the primary source of vitamin A for calves at birth, and they rely on it to establish vitamin A stores. Liver vitamin A concentrations are a sensitive indicator of vitamin A status because this is the primary vitamin A reserve in the body, and

concentrations are responsive to changes in dietary vitamin A intake. Contrary to our initial hypothesis, a cow with adequate liver vitamin A stores at the time of calving does not ensure that the calf will also have adequate liver vitamin A stores. Both the cow's liver vitamin A stores and dietary vitamin A intake, especially in late gestation, will both contribute to the vitamin A content of colostrum. Therefore, vitamin A intake by the cow in late gestation may be key in ensuring the calf receives adequate vitamin A from colostrum. More research is needed to understand how much dietary vitamin A intake needs to be provided to the cow to ensure sufficient intake and vitamin A stores in the young calf.

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Figure 3.1. Correlation of cow plasma and liver retinol concentrations. Dashed line indicates the liver retinol concentration considered adequate for cows (300 $\mu\text{g/g DM}$).

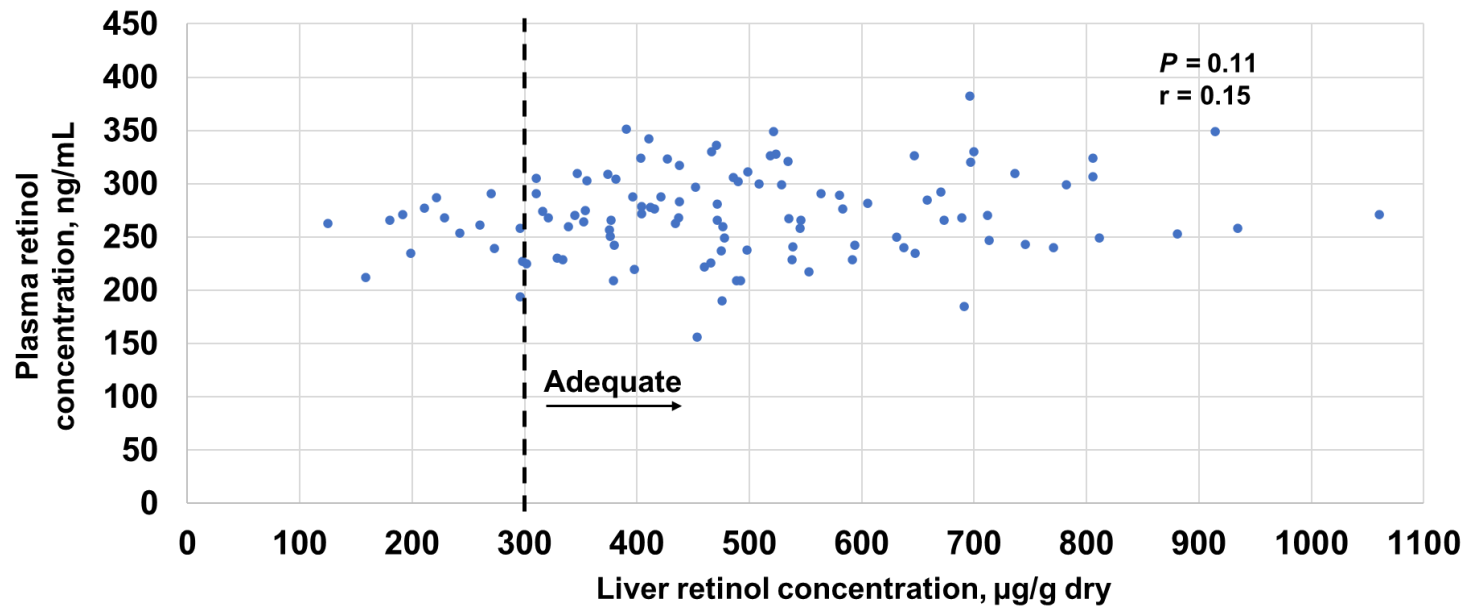


Figure 3.2. Correlation of calf plasma and liver retinol concentrations at 32 d of age. Dashed line indicates the liver retinol concentration considered adequate for calves at 32 d of age (100 $\mu\text{g/g DM}$).

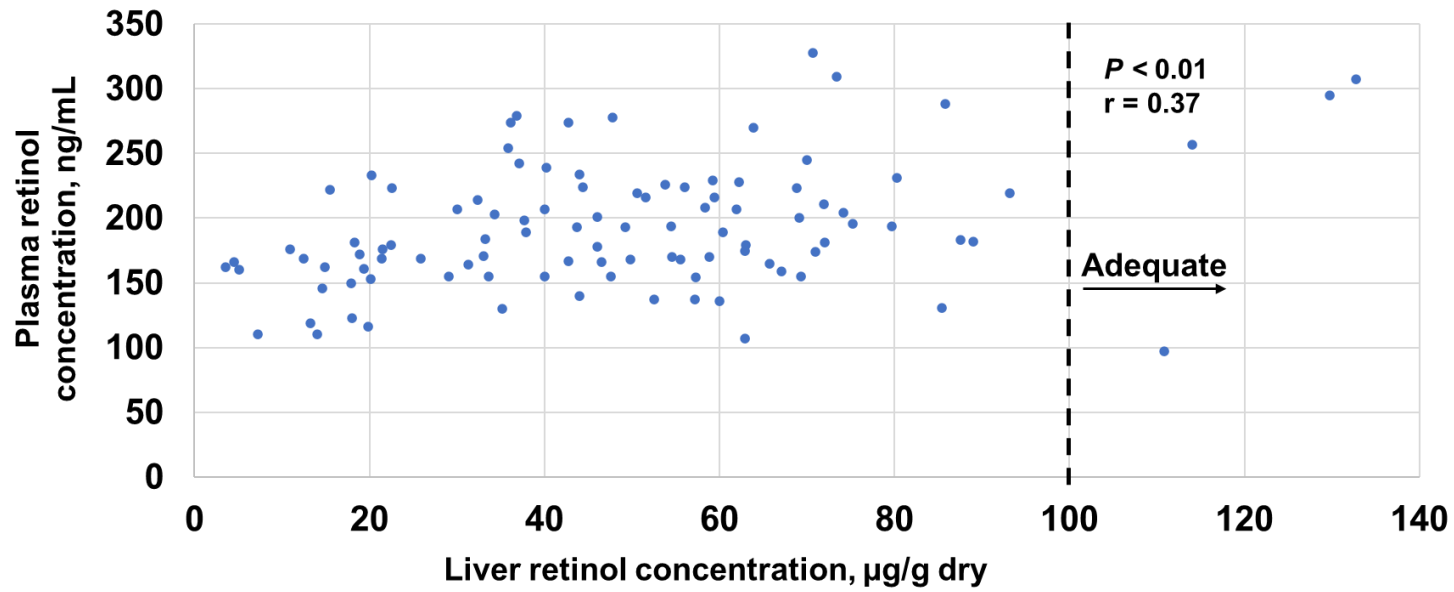
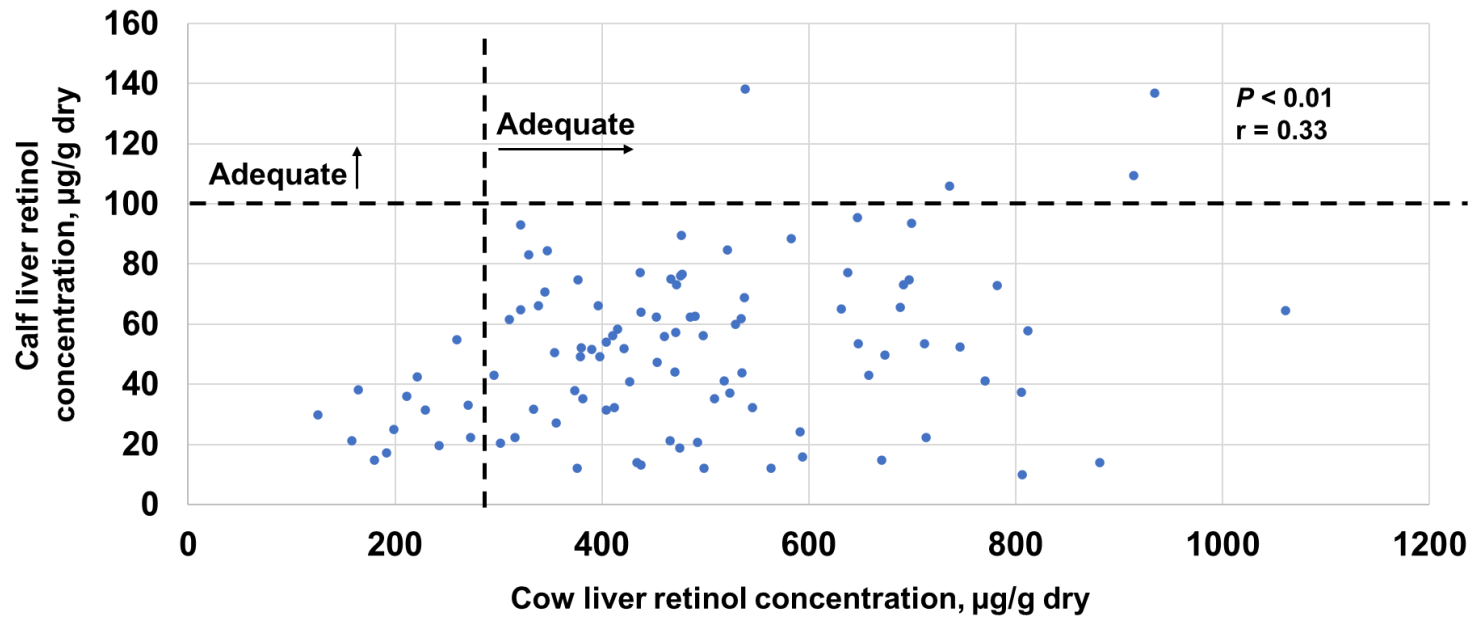


Figure 3.3. Correlation of calf liver retinol and cow liver retinol concentrations. Dashed lines indicate the liver retinol concentrations considered adequate for cows (300 µg/g DM) and calves at 32 days of age (100 µg/g DM).



**CHAPTER 4 - EFFECT OF VITAMIN A
SUPPLEMENTATION DURING GESTATION ON VITAMIN
A STATUS OF BEEF COWS AND THEIR CALVES
MANAGED IN THE DRYLOT**

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Abstract

Cows fed diets consisting primarily of stored forages and concentrates may be at risk for vitamin A deficiency because these feedstuffs are low in β -carotene. Furthermore, dietary vitamin A concentrations of the cow during late gestation will affect vitamin A concentrations in colostrum, which calves rely on at birth to supply vitamin A. The objective of this study was to evaluate how level of vitamin A supplementation from mid-gestation to early lactation affected cow, and subsequently, their calf's liver retinol concentrations. Multiparous beef cows ($n = 54$) were stratified by body condition score and previous source and assigned to pen ($n = 9$); 3 pens were assigned to each of the following treatments: current NASEM recommendation (31,000 IU/d; 1X), 3 times (93,000 IU/d; 3X), or 5 times (155,000 IU/d; 5X) the current NASEM recommendation for supplemental vitamin A. Cows were limit-fed a diet consisting of wheat straw, corn

silage, and wet distillers grains. Liver biopsies were collected on cows 24 d before treatment initiation, d 40 and d 81 of supplementation, and both cows and calves were sampled 32 d post-calving (165 ± 22 d SD of supplementation). No differences ($P = 0.86$) in initial cow liver retinol (mean $186 \mu\text{g/g DM}$) were observed between treatments. A significant treatment \times day interaction ($P < 0.01$) was observed for cow liver retinol. On d 40, cows in 1X had liver retinol concentrations ($178 \mu\text{g/g DM}$) that were not different ($P = 0.12$) from 3X ($213 \mu\text{g/g DM}$) but lower ($P = 0.02$) than 5X ($241 \mu\text{g/g DM}$), while 3X and 5X did not differ ($P = 0.21$). Liver retinol on d 81 was lower ($P < 0.05$) in 1X ($189 \mu\text{g/g DM}$) compared to 3X ($334 \mu\text{g/g DM}$) and 5X ($412 \mu\text{g/g DM}$), which did not differ ($P = 0.20$). For cow liver retinol post-calving, 1X ($187 \mu\text{g/g DM}$) was lower ($P < 0.05$) than 3X and 5X, and 3X ($454 \mu\text{g/g DM}$) was lower ($P < 0.05$) than 5X ($674 \mu\text{g/g DM}$). Liver retinol concentrations of 1X cows remained below adequate reference ranges ($300\text{--}700 \mu\text{g/g}$ of DM) throughout the study, whereas 3X and 5X were elevated into the adequate range by d 81. Calf liver retinol concentration also differed among treatments ($P = 0.01$), as calves of cows in 1X had lower ($P < 0.05$) liver concentrations than 3X and 5X calves which did not differ ($P = 0.12$). Liver retinol concentrations considered adequate for calves at 32 days of age ($100\text{--}350 \mu\text{g/g}$ of DM) were not observed in 1X calves ($51 \mu\text{g/g DM}$) but were observed in calves from 3X and 5X cows (119 and $165 \mu\text{g/g DM}$, respectively). Supplementing cows with $93,000$ IU/d of vitamin A for 165 days brought liver retinol concentrations of cows and their calves up within adequate reference ranges.

Introduction

Grazing of green pasture in the spring and summer helps cows build up liver stores that can be used during the fall and winter, as the amount of β -carotene, a vitamin A precursor, is sizable in fresh green forages (Puls, 1994; Weiss, 1998). Because β -carotene content of forages is directly related to their green color (Puls, 1994), forages that do not appear very green (e.g. dormant range, corn residue, straw, and sun-bleached hay) will be low in vitamin A. Green hay contains 14 times less β -carotene than fresh green forage, while ensiled feeds such as corn silage have roughly twice the vitamin A concentration found in green hay (Pickworth et al., 2012). Moreover, grains and grain byproducts contain little β -carotene (Pickworth et al., 2012); hence, it is likely cows fed diets consisting primarily of stored forages and concentrates may become deficient in vitamin A if vitamin A supplementation is not provided.

Vitamin A is essential in developing disease resistance in the young calf. Calves are born deficient in vitamin A because placental transfer of fat-soluble vitamins is minimal (Malone, 1975). Vitamin A is an important regulator of immune function, thus, a deficiency could result in the calf becoming immunocompromised (Jones et al., 1962; Semba, 1998). In the calf, vitamin A is particularly important for immune system maturation (Nonnecke et al., 1999). Colostrum is a good source of vitamin A as evidenced by studies indicating increased plasma vitamin A concentrations of calves after colostrum ingestion shortly after birth (Zanker et al., 2000; Puvogel et al., 2008). Stewart and McCallum (1938) suggested that dairy calves receiving colostrum low in vitamin A were more susceptible to illness, and eventually death, from white scours and secondary

infections, indicating that vitamin A in colostrum plays a role in protecting calves against disease.

The current recommendation for supplemental vitamin A of gestating beef cows was established in the 1970's (NRC, 1976) and is 2,800 IU/kg DM of feed, or approximately 60 IU/kg BW (NASEM, 2016). This recommendation is based on a mere 2 studies conducted with gestating beef cows (Guilbert and Hart, 1935; Meacham et al., 1970). It is unclear how the recommendation is derived from these studies, and no data are available on supplemental vitamin A needs of modern beef cows.

Guilbert and Hart (1935) briefly summarized observational data collected on 7 gestating beef heifers from a previous study (Guilbert and Hart, 1934) fed diets essentially devoid of vitamin A to deplete their liver stores. Two heifers received the basal diet of dried beet pulp and wheat straw, and the rest of the heifers were supplemented with alfalfa hay. Two of the heifers receiving the basal diet and alfalfa hay showed no signs of deficiency, but their calves developed night blindness (60 days of age) and/or diarrhea (7 to 60 days of age) and symptoms disappeared after treatment with cod liver oil, which is a source of vitamin A. In fact, all five calves born in this study developed severe diarrhea within the first few weeks of life: one died at 6 d of age following treatment with cod liver oil for 3 d, and one recovered without treatment. These data suggest the calf is at greater risk of vitamin A deficiency than the cow when the cow consumes feedstuffs low in vitamin A.

Subsequent experiments utilizing all but 1 heifer from their previous study (Guilbert and Hart, 1934) along with 4 additional animals (at least 1 bull) supplemented alfalfa hay or dehydrated alfalfa meal to provide β -carotene and suggested 26 to 33 μ g

carotene/kg BW as a minimum requirement for preventing night blindness in cattle (Guilbert and Hart, 1935). This minimum recommendation equates to 10.4 to 13.2 IU/kg BW as 1000 μ g of β -carotene is equal to 400 IU of vitamin A (McDowell, 2000). When discussing the requirement for reproduction, Guilbert and Hart (1935) noted that females were not able to produce a healthy calf when fed carotene deficient rations long-term and provided carotene supplementation in the form of alfalfa hay, which provided an additional 9.5 to 15 mg of carotene (3,800 to 6,000 IU) per day to their diet. This reiterates the fact that the minimum vitamin A needs of the gestating cow are not sufficient to meet vitamin A needs of her young calf.

Meacham et al. (1970) conducted two experiments where gestating beef cows were supplemented 16,000 IU/d to a grass silage and hay-based diet for either 55 (Exp. 1; 255 cows supplemented) or 106 d (Exp. 2; 157 cows supplemented) prior to calving, and then 40,000 IU/d during lactation. The basal diet was reported to have the equivalent of approximately 5,000 IU/kg, meaning cows were consuming an additional 46,000 and 72,000 IU/d from the basal diet in gestation and lactation, respectively. The BW of the cows was not reported, but if cow BW is assumed to be 478 kg based on historical BW of cows during the 1970's (McMurry, 2009), total vitamin A intake during gestation would have equated to 96 and 130 IU/kg BW for the non-supplemented and supplemented treatments, respectively. The supplement amount of 16,000 IU/d alone would have provided 29 IU/kg BW, which is nearly triple the amount suggested by Guilbert and Hart (1935) and almost half of the current NASEM (2016) recommendation of 47 IU/kg BW. When supplementation in gestation was 55 d in length, death losses of calves in experiment 1 were 16.5% in the non-supplemented group and were almost double that of

the supplemented group (8.8%). However, in trial 2, when they moved the calving season from January to March and extended the period of supplementation during gestation, death losses of calves were minimal and not different between treatments (mean 3%). This occurred even though the cows in the supplemented and non-supplemented groups in experiment 2 had greater loss of and lower overall vitamin A reserves at the end of the experiment than the non-supplemented cows in experiment 1. The lower death loss was attributed to less adverse weather conditions with the later calving season; however, it is possible that extending supplementation from 55 to 106 d before calving could have impacted the amount of vitamin A in colostrum while not being sufficient to maintain cow liver reserves.

Colostrum vitamin A concentration will be dictated by both the cow's vitamin A status and dietary vitamin A intake, with the diet contributing 60% of the vitamin A in the colostrum and liver stores the other 40% (Branstetter et al., 1973; Tomlinson et al., 1974). In calves at 28 d of age, Nezvesky et al. (1950) observed greater liver vitamin A concentrations in calves fed colostrum from cows supplemented for 30 d before calving with 1,000,000 IU/d vitamin A compared to calves receiving colostrum from cows receiving no vitamin A supplement. Additionally, they noted that within the group of calves fed colostrum from dams receiving no vitamin A supplement, liver concentrations of vitamin A at 28 d of age tended to be greater in calves whose dams were provided with vitamin A supplementation 30 d before calving. This highlights the importance of the dietary concentration of vitamin A during late gestation to supplying the calf with vitamin A through colostrum and suggests that cow liver reserves alone may not be sufficient to meet her calf's vitamin A needs.

Drylot cow diets which utilize brown, low quality roughages along with grain or grain byproducts, will have very low vitamin A concentrations due to their low β -carotene content. Silages could serve to increase dietary vitamin A concentrations, but storage time and conditions, as well as silage management, result in variation in β -carotene content (Kalac and McDonald, 1981).

The objective of this study was to determine the effect of 3 different supplemental vitamin A levels on liver retinol concentrations (a measure of vitamin A status) of gestating beef cows and their calves in a drylot-only production system. Our hypothesis was that current NASEM recommendations for supplemental vitamin A would not be sufficient for gestating cows fed stored forages and concentrates for an extended period to meet the vitamin A needs of their calves as indicated by liver retinol concentrations.

Materials and Methods

Cattle and feeding

Research protocols were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee.

This experiment was conducted in the feedlot located at the Panhandle Research and Extension Center in Scottsbluff, Nebraska. Multiparous cross-bred beef cows ($n = 54$) in mid-gestation (average of 125 d from calving) managed in a drylot system for ≥ 1 yr were used. Prior to the start of the study cows had been receiving 31,000 IU/d of supplemental vitamin A which is consistent with the NASEM (2016) recommendation for supplemental vitamin A for a gestating cow with a body weight of 546 kg that consumed 2.0% of body weight in DM per day. Cows were stratified by body condition

score (BCS) and time in the system and assigned to 1 of 9 pens. Pens were then randomly assigned to 1 of 3 treatments (n = 3/treatment). Treatments consisted of 3 vitamin A supplementation levels: 31,000 (1X), 93,000 (3X), and 155,000 (5X) IU/d. Basal dietary levels of vitamin A were on average 21,000 IU/d (Table 4.1). Vitamin A was provided as retinyl acetate (International Nutrition; Omaha, NE) and delivered to cows in the diet daily via a micronutrient machine (Micro Technologies; Amarillo, TX). Throughout the experiment cows were limit-fed a ration once daily to meet nutrient requirements during gestation and lactation that consisted of wheat straw, corn silage, and wet distillers grain plus solubles (WDGS; Table 4.1). The proportion of corn silage and WDGS in the diet was increased during lactation to increase energy density of the diet during lactation (Table 4.1).

Sample collection and analyses

Ingredient samples were collected monthly and were stored at -20°C until time of analysis. Samples were sent to a commercial laboratory (Dairyland Laboratories, Inc.; Arcadia, WI) for analysis of DM, OM, NDF, ADF, and EE. Ingredient samples were collected on d 81 and sent to a diagnostic lab (Michigan State University Veterinary Diagnostic Lab; Lansing, MI) for β -carotene analysis using ultra performance liquid chromatography (AOAC, 2011; method 2001.13). Beta-carotene concentration of each ingredient was used to estimate basal dietary vitamin A concentration, assuming 1000 μg of β -carotene is equal to 400 IU of vitamin A (McDowell, 2000).

An experimental timeline is displayed in Figure 4.1. On d 24 before treatment initiation, a liver biopsy were collected for a baseline measure of liver vitamin A

concentration. Additional liver samples were collected on cows on d 40 (85 ± 15 d SD pre-calving) and d 81 (44 ± 15 d SD pre-calving) of supplementation, and both cow and calves were sampled 32 ± 7 d SD post-calving (165 ± 22 d SD of supplementation). For the post-calving time point, cow-calf pairs were sampled in 3 groups to target an average sampling calf age of 32 d. The distributions of treatments represented within calving group is displayed in Table 4.2.

Liver biopsies were collected using the method of Engle and Spears (2000) for determination of liver vitamin A. Liver samples were rinsed with phosphate buffered saline (PBS) solution and collected into 2-mL microcentrifuge tubes, placed in a cooler with dry ice, and transported to the lab where they were stored at -80°C until time of vitamin A analysis. Liver samples were homogenized via pulverization using a cryogenic grinder (Freezer/Mill 6870D; SPEX Sample Prep, Metuchen, NJ) prior to vitamin A analysis. On day of analysis, liver samples were retrieved from storage and immediately placed in liquid N. Tubes were then removed from liquid N and broken to remove tissue. Tissue was quickly placed in a weigh boat and placed on dry ice until transfer into cryogenic grinding vials resting in liquid N. A pre-cooled stainless steel rod was placed in each vial, and vials were then capped with a room-temperature stainless steel plug before placement into the cryogenic grinder. Once ground, liver samples were transferred from the vials into aluminum weigh boats sitting on dry ice. Samples were then transferred into 5-ml microcentrifuge tubes and stored on dry ice until weighing. Pulverized samples were weighed into 50-mL falcon tubes and placed on dry ice.

Vitamin A concentrations in liver samples were determined utilizing a portable fluorometer (iCheck FluoroTM, BioAnalyt GmbH, Teltow, Germany). Extraction vials

designed for use with the fluorometer contain 98% hexane, 1% isopropanol, and 1% ethanol. Distilled water was added to liver samples to achieve a dilution factor (DF) of approximately 100 or 50 for cow and calf liver samples, respectively. Falcon tubes were then capped and shaken by hand for 1 min. Immediately afterwards, 500 μ L of solution was injected into extraction vials and shaken intensively for 10 s. Extraction vials were then centrifuged at 650 rpm for 10 min to achieve phase separation. Vials were inserted into the fluorometer and vitamin A concentration was measured. A pig liver was obtained and multiple subsamples were collected to test this extraction procedure prior to sample analysis. Accuracy of the procedure was verified by sending subsamples from this liver to a commercial laboratory (Michigan State University Veterinary Diagnostic Lab; Lansing, MI) where they were analyzed for vitamin A concentration via ultra performance liquid chromatography using modified procedures from Schmitz et al. (1991) and Rettenmaier and Schuep (1992). All readings were expressed as μ g retinol equivalents (RE)/L liver. Once analyzed for vitamin A content, the remainder of the liver sample from each animal at each time point was dried for 24 h at 100 °C in a forced-air oven to determine DM. Vitamin A concentration of liver are reported as μ g retinol/g DM.

Statistical Analysis

Liver retinol concentration of cows and calves were analyzed using the MIXED procedure of SAS. Pen was considered the experimental unit. For cow liver retinol concentration, day was considered a repeated measure with subject equal to pen and initial liver vitamin A concentration was used as a covariate. The covariance structure selected was unstructured. A covariate was used because cows enrolled on to study

entered the confinement system in three different years, resulting in differences in initial liver concentrations. Cows that had been in the system the longest (> 2 yr) had a mean initial liver retinol concentration of 107 $\mu\text{g/g DM}$ (minimum 36 $\mu\text{g/g DM}$; maximum 281 $\mu\text{g/g DM}$). Cows that had spent the least amount of time in the system (1 yr) had an initial raw mean liver retinol concentration of 448 $\mu\text{g/g DM}$, with minimum and maximum concentrations of 122 and 651 $\mu\text{g/g DM}$, respectively. The raw mean of the initial liver retinol concentration of the group of cows who were intermediate in time spent in the system was 120 $\mu\text{g/g DM}$ with minimum and maximum of 77 and 203 $\mu\text{g/g DM}$, respectively. If a cow had a liver retinol concentration absent from a time point, her initial liver concentration was removed at that time point and not included in the covariate. The covariate adjustment did not drastically change the cow liver retinol concentrations reported; concentrations were never adjusted more than 34 μg from the arithmetic mean (Appendix A). Fixed effects for cow model included treatment, day, and the interaction. The model for calf liver vitamin A included treatment as a fixed effect. Significance was declared at $P \leq 0.05$.

Results and Discussion

Liver retinol concentration was selected as the indicator of cow and calf vitamin A status because it is a more sensitive measure status compared to plasma retinol concentrations. Approximately 90% of total body vitamin A is stored in the liver and will increase as dietary vitamin A intake increases, but not in a linear fashion (Olson, 1984). As dietary vitamin A intake increases, storage efficiency will decrease (Olson, 1984). In contrast, plasma concentrations do not fluctuate (Blomhoff et al., 1992; Goodman, 1984)

unless liver stores become severely depleted (Dowling and Wald, 1958; Olson, 1984) or when liver storage capacity is exceeded (Olson, 1984).

No differences ($P = 0.86$) in initial cow liver retinol concentration (mean 186 $\mu\text{g/g DM}$; Figure 4.2) were observed among treatments. All cows were receiving the 1X supplemental vitamin A level for over a year before the study began, suggesting the current supplemental vitamin A recommendation of 31,000 IU/d was not enough to result in adequate liver retinol concentrations (300–700 $\mu\text{g/g DM}$; Puls 1994). The basis for this recommendation is not understood when assessing the 2 studies conducted with gestating beef cows (Guilbert and Hart, 1935; Meacham et al., 1970) that were used to establish it. Guilbert and Hart (1935) suggested 26 to 33 $\mu\text{g carotene/kg BW}$ (10.4 to 13.2 IU/kg BW) as a minimum requirement for preventing clinical deficiency symptoms in beef cows fed diets practically devoid of vitamin A for long periods of time. Meacham et al. (1970) collected liver samples before supplementation of 16,000 IU/d for 55 d during gestation, and again at 100 d of lactation after receiving 40,000 IU/d during lactation. They reported a small increase in cow liver vitamin A concentrations (249 to 296 $\mu\text{g/g}$ on a wet basis; 830 to 987 $\mu\text{g/g DM}$). Due to the timing of sampling, it is impossible to separate the impact of dietary vitamin A in gestation from that in lactation on liver retinol concentrations. In the current study, liver stores did not increase to adequate concentrations on the 1X treatment, suggesting that 31,000 IU/d may not be an appropriate recommendation for cows being managed on stored feedstuffs for a long period of time to maintain adequate liver vitamin A stores. The NASEM (2016) supplemental vitamin A recommendation does not account for differences in basal dietary vitamin A concentrations, and it is likely that this recommendation assumes cows

will be on green pasture for a large portion of the year and can build liver stores that can be used when dietary vitamin A intake is lower. Cows having no access to fresh green forage like the ones in the current study do not have the opportunity to build liver stores, and are getting very little vitamin A from the basal diet due to the types of ingredients (stored forages and concentrates) their diets are made from.

A significant treatment \times day interaction ($P < 0.01$) was observed for cow liver retinol concentrations (Figure 4.2). On d 40, cows in 1X had liver retinol concentrations (178 $\mu\text{g/g DM}$) that were not different ($P = 0.12$) from 3X (213 $\mu\text{g/g DM}$) but lower ($P = 0.02$) than 5X (241 $\mu\text{g/g DM}$), while 3X and 5X did not differ ($P = 0.21$). Liver retinol on d 81 was lower ($P < 0.05$) in 1X (189 $\mu\text{g/g DM}$) compared to 3X (334 $\mu\text{g/g DM}$) and 5X (412 $\mu\text{g/g DM}$), which did not differ ($P = 0.20$). For cow liver retinol post-calving, 1X (187 $\mu\text{g/g DM}$) was lower ($P < 0.05$) than 3X and 5X, and 3X (454 $\mu\text{g/g DM}$) was lower ($P < 0.05$) than 5X (674 $\mu\text{g/g DM}$). Liver retinol concentrations of 1X cows remained below adequate reference ranges (300–700 $\mu\text{g/g DM}$) throughout the study, whereas 3X and 5X were elevated into the adequate range by d 81 of supplementation. We did not observe any signs of vitamin A deficiency in any cow throughout the study, but others have observed symptoms of abortion, calves born prematurely, and night blindness in cows when liver vitamin A concentrations were less than 5 $\mu\text{g/g DM}$ (Wheeler et al., 1957; Swanson et al., 1968), so it appears that cow liver stores must be severely depleted before clinical symptoms appear. The data suggests that cows with initially low liver retinol stores needed to be fed 93,000 IU/d (3 times the NASEM recommendation) of vitamin A to achieve adequate liver retinol concentrations, as defined by Puls (1994) by d 81 of supplementation. However, this amount did appear to result in continuously

increasing liver stores, so it is unlikely cows will need this level of supplementation long-term to maintain adequate liver vitamin A stores.

Calf liver retinol concentration also differed among treatments ($P = 0.01$; Figure 4.3), as calves of cows in 1X had lower ($P < 0.05$) liver concentrations than 3X and 5X calves which did not differ ($P = 0.12$). Liver retinol concentrations considered adequate for calves at 32 days of age (100–350 $\mu\text{g/g}$ of DM; Puls 1994) were not observed in 1X calves (51 $\mu\text{g/g}$ DM) but were observed in calves from 3X and 5X cows (119 and 165 $\mu\text{g/g}$ DM, respectively). Despite cows on the 3X and 5X treatment reaching adequate liver retinol status by d 81, only 60% of the 3X calves and 80% of the 5X calves reached liver retinol concentrations greater than 100 $\mu\text{g/g}$ DM.

It is not clear how the adequate reference range for calf liver retinol concentrations was determined; however, the 100 $\mu\text{g/g}$ DM threshold seems reasonable from a calf health standpoint. Swanson et al. (2000) observed less incidence of diarrhea and hyperthermic rectal temperatures in dairy calves from d 4 to 28 of age when supplemental vitamin A level increased from 2,300 IU to 44,000 IU/kg DM. When concentrations of less than 9,000 IU/kg DM were provided, decreases in liver retinol concentrations were observed, while calves receiving 9,000 IU/kg DM had essentially maintained liver retinol concentrations at 75 $\mu\text{g/g}$ DM through d 28. Another study conducted with 28 dairy calves observed 5 cases of mild diarrhea (18%) from birth to 28 d of age, with liver vitamin A levels at 28 d of age ranging from 60 to 80 $\mu\text{g/g}$ DM (Nezvesky et al., 1950). It should be noted that liver vitamin A levels in both studies were reported on a wet basis and were converted to a dry basis assuming a 30% liver DM. These studies suggest that perhaps the threshold before seeing adverse health effects in

young calves may be lower than 100 µg retinol/g DM; however, calves in both of these studies were individually housed and Swanson et al. (2000) included antibiotics in the milk replacer, so calf management could have played a significant role in the disease incidence observed at liver vitamin A concentrations less than 100 µg/g DM. In the current study, 11 calves out of 50 born live were treated for illness, with 64% of the calves treated being from 1X and 3X treatment groups. No statistical comparisons of calf morbidity were conducted due to the low number of animals. Overall, our results demonstrate for cows fed stored feeds long-term, supplementing cows with the current NASEM (2016) recommendation for vitamin A will not result in their calf's liver vitamin A concentrations being within the adequate reference range.

Both cow liver concentrations and dietary vitamin A intake will impact calf vitamin A status, because 60 and 40 percent of vitamin A contributions to colostrum come from diet and cow liver stores, respectively (Branstetter et al., 1973; Tomlinson et al., 1974). Feeding less than 93,000 IU/d to cows who were of adequate status may not result in sufficient vitamin A concentrations in colostrum for the calf, given that dietary vitamin A contributes slightly more to colostrum than cow liver stores. Further investigation is warranted for the length of time and amount of vitamin A supplementation cows should be provided prior to calving to achieve adequate liver retinol concentrations in young calves.

Supplementing cows with 93,000 IU/d of vitamin A for 165 days brought liver retinol concentrations of cows and their calves up within the middle of the adequate reference range for cows. When other published vitamin A recommendations for ruminants are scaled to a 546 kg gestating beef cow, the dairy NRC (2001) supplemental

recommendation would be about 60,000 IU/d, but this recommendation is focused on cow health and productivity rather than optimal calf vitamin A status. The small ruminant NRC (2007) and ruminant CSIRO (2007) recommendations are 89,600 and 88,600 IU/d, respectively, which are described as total rather than supplemental vitamin A intake recommendations. These values agree quite closely with what we would suggest the supplemental vitamin A intake to be for cows receiving a diet with low basal vitamin A.

Conclusion

The calf is at greatest risk of vitamin A deficiency when cow vitamin intake is low. These data suggest feeding the NASEM supplemental vitamin A recommendations to cows fed stored feeds long term does not result in adequate beef cow or calf liver retinol concentrations. Cows in the current study who received the NASEM supplemental recommendation (31,000 IU/d) had very little change in liver retinol concentrations from d -24 (192 $\mu\text{g/g DM}$) to 165 (187 $\mu\text{g/g DM}$) of supplementation. In contrast, liver retinol concentrations increased over time when supplementing either 93,000 IU/d or 155,000 IU/d to cows. Supplementing 93,000 IU/d for 125 days prior to calving and 32 days post calving resulted in cows having liver retinol concentrations that increased from deficient to middle of the adequate range and resulted in most of their calves having adequate liver retinol concentration at 32 d of age. More research is needed to understand the exact amount of supplemental vitamin A required to maintain cow liver retinol concentrations in the adequate range while also ensuring adequate concentrations in the colostrum for the calf, and how these supplemental vitamin A needs would differ in other production systems.

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Table 4.1. Composition of diets and amounts offered to cows.

Ingredient	Gestation	Lactation	Lactation
	d 0 to 107	d 108 to 153	d 154 to 165
% of diet DM			
Wheat straw	54.5	44.5	37.5
Corn silage	25.0	32.0	37.0
WDGS ¹	20.0	23.0	25.0
Mineral supplement ²	0.625	0.500	0.521
Vitamin A supplement ³	0.011	0.009	0.009
% of DM			
Dry matter	64.01	60.69	58.66
Organic matter	91.59	92.38	92.63
Crude protein	12.38	12.72	12.92
Neutral detergent fiber	58.77	57.03	55.81
Acid detergent fiber	37.28	36.51	35.81
Ether extract	3.61	3.79	3.47
Vitamin A, IU/kg ⁴	1586	2010	2311
kg of DM/d			
Diet	9.1	11.4	11.4

¹Wet distillers grains plus solubles.

²Composition: 26% Ca, 12% Mg, 6% Na; 5,700 mg/kg Zn, 4,200 mg/kg Mn, 2,000 mg/kg Cu, 105 mg/kg I, 26 mg/kg Se, 5 mg/kg Co.

³Provided either 31,000 (1X), 93,000 (3X), or 155,000 (5X) IU/d vitamin A as retinyl acetate.

⁴Individual ingredients analyzed for β -carotene; calculated as 1000 μ g of β -carotene = 400 IU vitamin A.

Table 4.2. Distribution of cow-calf pairs from each treatment within calving group (1, 2, 3) sampled on 165 ± 22 d SD of supplementation. Cows were placed into groups based on calving date to target an average age of 32 d for calves at time of sampling.

Calving group	Number of pairs per treatment ¹			<u>Totals</u> ²
	<u>1X</u>	<u>3X</u>	<u>5X</u>	
1	10	7	8	25
2	7	7	5	19
3	1	3	3	7

¹Vitamin A supplementation level. 1X = 31,000 IU/d (current NASEM recommendation), 3X = 93,000 IU/d, and 5X = 155,000 IU/d.

²Three pairs not sampled; one cow did not calve (5X), and two cows were removed from study because of health reasons (3X and 5X).

Figure 4.1. Experimental timeline. Supplementation began on Day 0 (average 125 days before calving). Liver biopsies were collected on cows on d -24, d 40 (85 days pre-calving), d 81 (44 days pre-calving), and d 165 of supplementation (32 d post-calving). Liver biopsies were conducted on calves at d 165 (32 d of age).

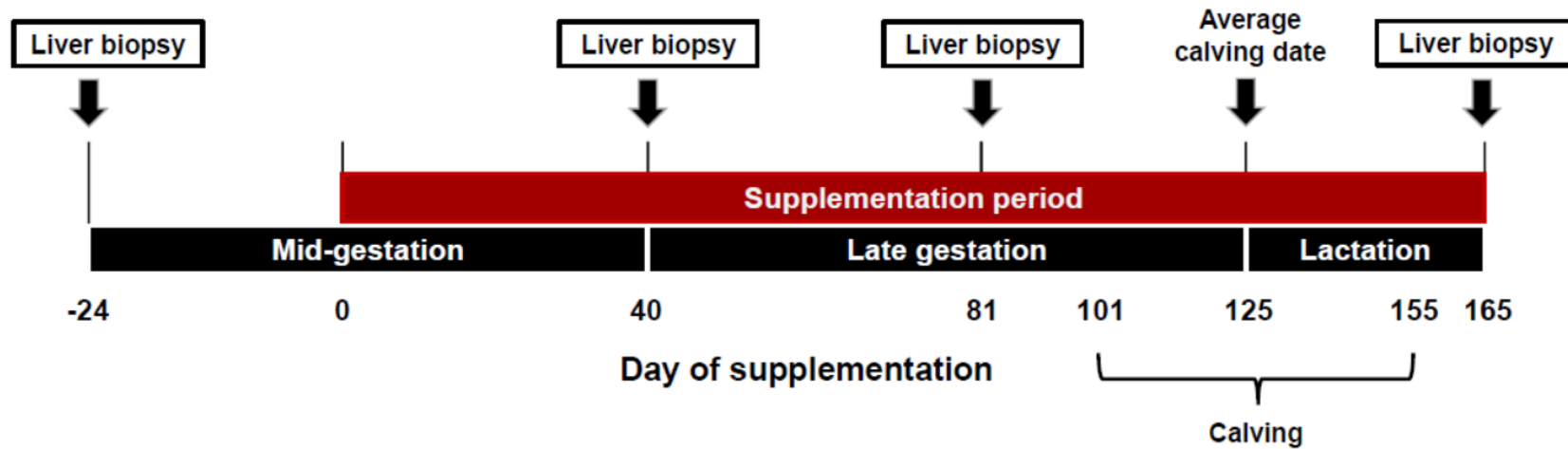
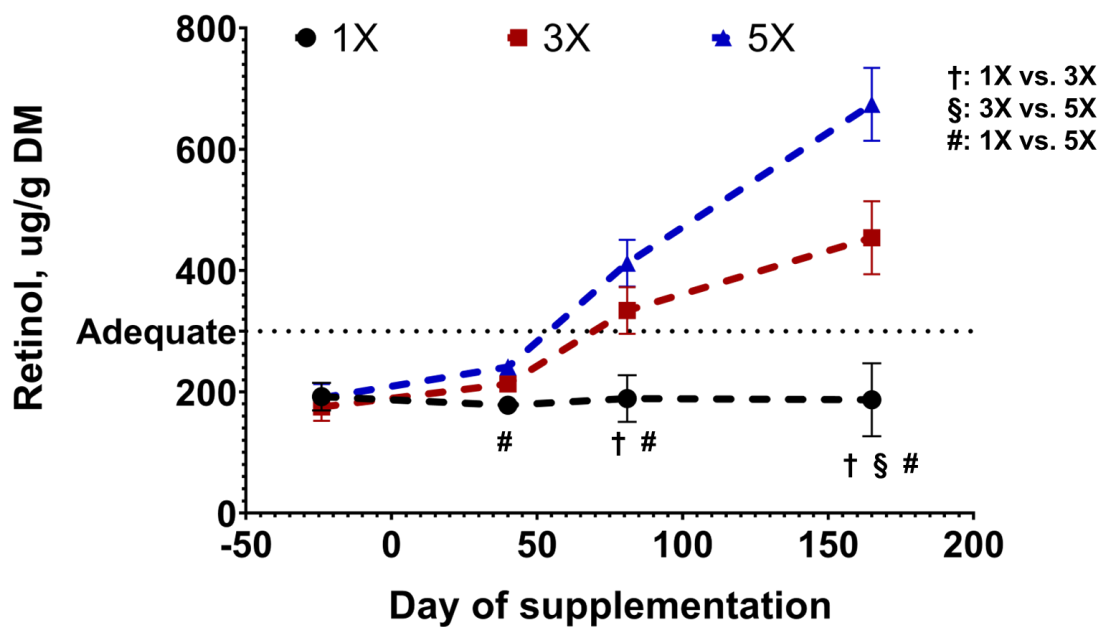
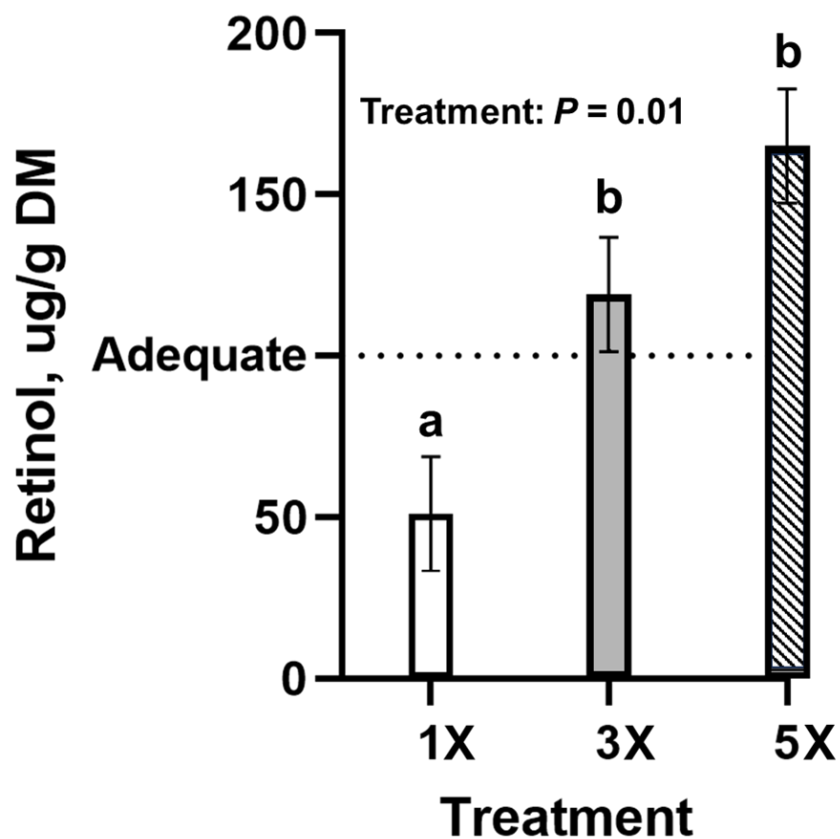


Figure 4.2. Effect of supplemental vitamin A level [1X = 31,000 IU/d (current NASEM recommendation), 3X = 93,000 IU/d, and 5X = 155,000 IU/d] on cow liver retinol concentrations. Dashed line indicates the liver retinol concentration considered adequate for cows (300 $\mu\text{g/g DM}$; Puls, 1994). † § # Significant difference of $P \leq 0.05$.



Treatment \times time interaction significant ($P < 0.01$).

Figure 4.3. Effect of cow supplemental vitamin A level (1X = 31,000 IU/d; 3X = 93,000 IU/d; 5X = 155,000 IU/d) on calf liver retinol concentration at 32 ± 7 days of age. Dashed line indicates the liver retinol concentration considered adequate for calves at 32 days of age (100 $\mu\text{g/g DM}$; Puls, 1994). Bars lacking a common letter differ ($P \leq 0.05$).



Main effect of treatment significant ($P = 0.01$).

**APPENDIX A - COMPARISON OF LIVER VITAMIN A
CONCENTRATIONS WITH AND WITHOUT COVARIATE
ADJUSTMENT**

Table A.1. Comparison of mean liver vitamin A concentrations by day and supplementation level (1X = 31,000 IU/d; 3X = 93,000 IU/d; 5X = 155,000 IU/d) that were calculated arithmetically or with the covariate adjustment of initial liver retinol concentrations. Means are expressed as μg retinol/g DM.

Time point¹	1X		3X		5X	
	<u>Raw²</u>	<u>Adjusted³</u>	<u>Raw</u>	<u>Adjusted</u>	<u>Raw</u>	<u>Adjusted</u>
d 40	199	178	200	213	274	240
d 81	185	175	353	332	453	425
d 165	176	201	414	447	670	656

¹Day of supplementation.

²Arithmetic means of liver vitamin A concentration at a given time point within treatment.

³Means adjusted using covariate.