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Feeding Different Iodine Sources to Dairy Cows: Effects on Colostrum Production and Growth and Health of Their Calves

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FEEDING DIFFERENT IODINE SOURCES TO DAIRY COWS: EFFECTS ON COLOSTRUM PRODUCTION AND GROWTH AND HEALTH OF THEIR CALVES

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ABSTRACT

FEEDING DIFFERENT IODINE SOURCES TO DAIRY COWS: EFFECTS ON COLOSTRUM PRODUCTION AND GROWTH AND HEALTH OF THEIR CALVES

by

Kayla Renee Johnston

University of New Hampshire, September, 2022

Limited research exists on supplementing seaweeds to prepartum cows and its effects on colostrum production and development of their calves in-utero and after birth. The high iodine (**I**) concentration of seaweeds is concerning, and previous research have shown that excess I supplementation to ewes inhibits immunoglobulin G (**IgG**) absorption in their lambs. The objectives of this study were: (1) evaluate the effects of incremental amounts of *Ascophyllum nodosum* (**ASCO**) meal supplementation (0, 57, and 113 g/d) to prepartum cows on colostrum production and subsequent growth, health, and blood concentrations of glucose, β-hydroxybutyric acid (**BHBA**), and thyroid hormones (**TH**) of their offspring after birth, and (2) compare ASCO meal versus a common I source [i.e., ethylenediamine dihydroiodide (**EDDI**)] on the same variables under objective 1. Forty Holstein dairy cows were blocked by lactation number and expected calving date and assigned to 1 of 4 treatments in a randomized complete block design. Treatments included: (1) EDDI supplemented to meet the National Research Council (**NRC**) 2001-recommended concentration of I [i.e., 0.5 mg I/kg of dry matter intake (DMI); control diet (**CON**)], CON plus 57 g/d of ASCO meal [low level (**LO**)], CON plus 113

g/d of ASCO meal [high level (**HI**)], and CON plus EDDI (124.8 mg/d) supplemented to match the amount of I provided by HI (**EDDI** diet). Iodine sources were top-dressed and manually mixed into the total-mixed ration (**TMR**) daily. Single degree freedom contrasts were used to statistically analyze the effects of adding incremental amounts of ASCO meal to the diet, as well as to compare HI versus EDDI.

Within approximately 1 h of calving, colostrum was harvested from cows and weighed. Colostrum was analyzed for fat, protein, total solids, I, and IgG. Forty-one calves averaging (mean ± standard deviation) 43. 9 ± 4.54 kg body weight (**BW**) were assigned to their dams' respective treatments and blocks, but they did not receive ASCO meal or EDDI. Calves remained on the study for (mean ± standard deviation) 56 ± 5 d. At birth, all calves were fed 750 g [dry matter (**DM**) basis] of colostrum replacer (**CR**), followed by 700 g CR 6 h after birth. At 24 h old, calves were offered 338 g (DM basis) of milk replacer (**MR**) (25% crude protein, 16% fat) twice daily until their final 7 d on experiment, where they were offered 338 g of MR once daily to facilitate weaning. Free choice textured starter (28% CP) and water were offered at 24 h of life until completion of the study. Blood samples were collected at 0 h and 24 h of age for IgG and TH analyses, and for TH at d 14, 28, and 56. Blood samples were taken weekly starting at wk 1 for BHB analysis. On d 5 of life, a xylose challenge was conducted by supplementing 0.5 g/kg BW of D-xylose in the MR, with blood samples taken before (0 h) and after D-xylose administration at 2, 4, 6, 8, and 12 h. Blood samples were analyzed for plasma xylose and glucose concentrations as proxies for absorption in the small intestine. Weekly measurements were recorded for BW, withers height, hip height, body length, heart girth, and hip width. Treatments did not affect colostrum yield, as well as protein, total solids, I, and IgG concentrations. Fat concentration of colostrum was greater in HI than EDDI cows, and there was

a tendency for fat concentration in colostrum to decrease linearly with ASCO meal supplementation. However, colostrum fat yield was not changed. Treatments had no effect on intake of calf starter, MR, or total DM intake, and average daily gain (**ADG**) or feed efficiency. Heart girth, body length, and hip width were similar across treatments. Initial BW was greater in HI compared with EDDI calves, and BW tended to decrease linearly with the addition of ASCO meal. Weekly hip height and final withers height tended to be greater in HI versus EDDI calves. Final hip height and weekly withers height were greater in HI than EDDI calves. Plasma concentration of weekly total T_4 responded quadratically to ASCO meal supplementation, with the lowest concentration occurring with the LO treatment. Weekly total T_4/T_3 ratio had a tendency to follow the same response as total T_4. All other TH (total T_3, and free T_3, and T_4) were unaffected by treatments. All calves obtained the minimum serum IgG recommendation for passive transfer (>10 g/L), and both 0 and 24 h serum IgG were not affected by treatments. However, there was a tendency for apparent efficiency of absorption to be lower in EDDI versus HI calves. Plasma concentration of weekly BHBA and final plasma BHBA responded quadratically to ASCO meal supplementation, with the lowest concentrations seen with LO. During the xylose challenge calves did not differ in D-xylose absorption; however, glucose absorption decreased linearly with ASCO meal supplementation. Overall, addition of ASCO meal to the dams' diet did not negatively impact calf growth and metabolism or colostrum production and composition.

CHAPTER 1:

REVIEW OF LITERATURE

Colostrum and its Importance in Newborn Calves

Colostrum Properties and Synthesis

Colostrum is the first secretion of cows after parturition, and is the first food of the neonatal calf (Pakkanen and Aalto, 1997). Colostrum contains a variety of nutrients including protein, carbohydrates, fat, minerals, and vitamins. However it is unique in that it contains a high concentration of growth and antimicrobial factors, and compounds that support the neonatal immune system such as immunoglobulin G (**IgG**) (Pakkanen and Aalto, 1997). Together, these nutrients and growth factors are vital in supporting the health and development of the neonatal calf. The formation of colostrum is referred to as colostrogenesis, and is a unique stage in mammary gland development where immunoglobulins from the dam's circulating blood are transferred to mammary gland secretions (Barrington et al., 2001). Colostrogenesis begins approximately 2-3 weeks prior to parturition and ceases before parturition (Brandon et al., 1971). It is important that colostrum produced by the dam is both high in IgG concentration, as well as produced in enough volume to feed the calf. This can be a challenge in breeds such as Holsteins that tend to produce colostrum that is lower than ideal in terms of the recommended IgG concentration of 50 g/L (Pritchett et al., 1991), despite producing adequate volume of colostrum (Foley and Otterby, 1978).

Importance of Colostrum for Calves

Neonatal calves have a limited ability to absorb immunoglobulins through placental tissues due to ruminants having a syneptiheliochorial placenta, which results in them being born hypogammaglobulinemic. Therefore, feeding calves at least 3.8 L, of high-quality (> 50 g/L IgG) colostrum during the first 24 h of life is critical to establishing the immune system of the neonate (Beam et al., 2009). Colostrum-derived immunity is referred to as "transfer of passive immunity" (**TPI)** and is determined by measuring the concentration of serum or plasma IgG in the calf within the first 7 d of birth (Lombard et al., 2020). Measurement of serum IgG also serves as a proxy for other colostrum-derived benefits such as hydration, thermal regulation, nutrient intake, and immune system function not related to IgG (Lombard et al., 2020). These benefits are provided by colostrum's high-nutrient concentration, specifically from proteins, fats, and growth factors (Shivley et al., 2018). Due to the intestinal barrier closing after 24 h and colostrum IgG concentration becoming more dilute over time, it is critical that colostrum be fed to the calf as soon as possible, ideally within 1 h after birth (Puppel et al., 2019).

Establishing immune function in the neonate is essential to survival and growth of the calf. Failure of a calf to obtain immunity from colostrum is referred to as "failure of passive transfer (**FPT**; Shivley et al., 2018), and until recently was indicated by a serum IgG measurement of <10 g/L (USDA, 1993). Failure of passive transfer is associated with increased calf mortality rate, with a study by Robison et al. (1998) noting that 44.2% of calves that died within 180 d of life had a serum IgG concentration of <12 g/L. Figure 1. shows the difference in calf mortality rates among calves with greater or less than 10 g/L serum IgG (USDA, 1993). However, new recommendations suggest that calf serum IgG concentrations of ≥25 g/L are

considered excellent absorption, followed by good absorption at 18.0-24.9 g/L, fair absorption at 10.0-17.9 g/L, and poor absorption at <10 g/L (Lombard et al., 2020). It is estimated that 90% of all Holstein heifer calves meet the previous serum IgG standard of 10 g/L (Shivley et al., 2018). However, data shows that the rate of morbidity (specifically scours and respiratory disease) in calves has only decreased by 3% from 1991 to 2014 (Lombard et al., 2020). Additionally, Urie et al. (2018b) found that calf survival probability and probability of not acquiring disease is increased in calves with serum IgG concentrations greater than 18 and 25 g/L, respectively (Figure 2a and 2b). The most effective method to improve TPI is to feed adequate amounts of high quality colostrum in a timely manner (Stott and Fellah, 1983; USDA 1993).

Weaver et al. (2000) stated that colostrum volume and IgG mass consumed by the calf are the most important factors influencing IgG absorption in the neonate. Excellent quality colostrum is defined as colostrum containing >50 g/L IgG (Shivley et al., 2018). Shivley et al. (2018) performed a field study involving 104 dairy operations and found a FPT rate of 12.1% out of 1,623 calves. Out of these calves with FPT, 46.2% were fed poor quality colostrum with IgG concentration ≤50 g/L. The study also found that 58.8% of calves with FPT were fed low volumes of colostrum, and 36.6% experienced delayed colostrum feeding. Analyses by Stott and Fellah (1983) found that colostrum IgG concentration is the primary factor in achieving successful TPI. When colostral IgG concentration and calf serum IgG concentration were plotted via regression, an r^2 of 0.77 was found for a 2 L feeding, and an r^2 of 0.74 was found for a 1 L feeding ($P < 0.01$ for both volumes), indicating that IgG absorption increased as more IgG was consumed (Stott and Fellah, 1983). General recommendations are to feed 4 L of colostrum containing >50 g/L IgG (Morin et al.,1997). While feeding colostrum as soon as possible and in

adequate amounts can be controlled by the farmer, controlling colostrum quality is more challenging.

Nutritional Influences on Colostrogenesis

Although some of the molecular and endocrine-level regulations of colostrogenesis are well understood, it is less understood how the diet of the dam affects colostrum quality, particularly in dairy cattle (Funston et al., 2010; Nowak et al., 2012). Some studies analyzing the effects of energy level and concentrate supplementation in prepartum diets did not find an effect on colostral IgG concentration (Nowak et al., 2012; Dunn et al., 2017), while other data shows prepartum diets supplying 150% of energy requirements to decrease colostral IgG concentration (Mann et al., 2016). However, it has been shown that feeding more rapidly degradable starch sources such as wheat grain rather than corn increased plasma total protein in dairy cows, as well as total solids, protein, and total IgG concentration in their colostrum than dams fed corn (Fatahnia et al., 2012). A meta-analysis has shown that an increase in omasal flow of nonammonia crude protein is correlated with an increase in milk protein yield (Huhtanen et al., 2010). Therefore, feeding more rapidly degradable starch sources in the dry period may increase microbial protein synthesis and allow for more amino acids to flow to the small intestine and be used in colostral protein synthesis (Huhtanen et al., 2010; Fatahnia et al., 2012). This theory was further supported by work involving feeding nicotinic acid (**NA**) to dams to improve colostrum quality; it has been found that feeding 48 g/d of NA increased colostral IgG concentration by 18% (Aragona et al., 2016). Nicotinic acid has vasodilative properties which allow for more blood flow to mammary epithelial cells and therefore more IgG transfer into colostrum (Aragona et al., 2016). Additionally, this is also supported by the microbial protein hypothesis, where NA

has been shown to increase the population of Entodinia protozoa in the rumen (Erickson et al., 1990). Entodinia help stabilize rumen pH and promote ruminal bacteria growth, which would allow for more microbial protein production to be used in synthesis of colostrum components (Erickson et al., 1990). Aragona et al. (2020) observed a linear increase in urine allantoin and total purine derivatives in response to increasing NA supplementation, which is indicative of increased microbial protein synthesis (Topps and Elliot, 1965), possibly from Entodinia. In the same study by Aragona et al. (2020), it was found that 48 g/d of NA produced the greatest colostral IgG and protein concentration compared to 0, 16, and 32 g/d NA supplementation. This linear increase in colostral IgG matches the linear increase in urinary purine derivatives with increasing NA supplementation, which suggests increased microbial protein synthesis. However, IgG yield, fat concentration and yield, and protein yield showed a positive quadratic response, with 16 and 32 g NA producing colostrum with the greatest IgG, protein, and fat yield, as well as fat concentration. Changes in colostral fat have been observed in other experiments where NA was fed to dairy cows (Belibasakis and Tsirgogianni, 1996), possibly because NA serves as a substrate for synthesis of NADP, a coenzyme for milk fat synthesis (Bauman and Davis, 1974; Aragona et al., 2020). Additionally, an increase in protozoa in the rumen may have increased neutral detergent fiber (**NDF**) digestibility (Horner et al., 1988) and subsequently acetate production in the rumen. As acetate is a precursor to milk fat synthesis, increased acetate concentrations could lead to increased colostral fat synthesis as well (Belibasakis and Tsirgogianni, 1996). Further research is needed to determine what dietary additives are capable of increasing fatty acid precursor availability and which are effective at increasing colostral fat. While effects of diet on colostrogenesis are still not well understood, it seems the most efficient dietary promoters of colostrum production are those that increase blood flow to the mammary

gland and that increase microbial protein synthesis. Increased microbial protein synthesis leads to more amino acid flow to the small intestine, which allows increased formation of colostral protein.

In-Utero Influences on Immunoglobulin G Absorption and Intestinal Development

Serum IgG at 24 h is strongly correlated with colostrum IgG intake as more than 50% of variation in calf 24 h serum IgG concentrations can be explained by mass of IgG consumed from colostrum (Kruse, 1970). Although colostral IgG concentration is a significant determinant in achieving passive transfer in the calf, calves can still fail to absorb IgG even when colostrum is fed correctly and is of high quality (Nowak et al., 2012; Hall et al., 2014). The opposite is also true; calves fed poor quality colostrum may be able to achieve passive transfer (Nowak et al., 2012; Hall et al., 2014). This was seen in a study by Hall et al. (2014) where calves born to dams supplemented with selenium (**Se**) yeast achieved higher TPI and IgG absorption despite being fed poorer quality colostrum than control calves. It is hypothesized that the immunological properties of Se, such as promoting pinocytosis and delaying apoptosis in the intestinal epithelium, help promote IgG absorption in the calf (Hall et al., 2014). More research is needed to determine how pinocytosis is influenced in-utero. Other studies hypothesize that the iodine (**I**) status of the dam can influence IgG absorption in the neonate. Boland et al. (2008) fed elevated I levels of 26 mg I/d to ewes for 3 wk prepartum and found their lambs to have lower serum IgG concentrations and apparent efficiency of absorption (**AEA**) than lambs born to control ewes. Even though IgG concentration of colostrum consumed by both control and high I lambs was 80 g/L, the excess I lambs had an average 24 h serum IgG concentration of 8.2 g/L, which is below the recommended 23 g/L for lambs (Boland et al., 2008). Iodine controls thyroid hormone (**TH**)

metabolism, therefore it is possible that improper TH production alters the metabolism in a way that inhibits IgG absorption in neonatal lambs, likely through altered gene expression of intestinal receptors (Boland et al., 2008, McGovern et al. 2015; 2017). Due to the effects of maternal protein intake on colostrum production, there is also interest on how maternal protein intake of dams affects IgG uptake in their calves. Calves born to dams fed a 14.2% crude protein (**CP**) diet tended to have 27.8% greater serum IgG concentration at 72 h after birth and higher serum IgG at 21 d of age than calves born to dams fed an 11.9% CP diet (Toghyani and Moharrery, 2015). IgG concentration of the maternal colostrum was not different among treatments, therefore the effects of IgG absorption in the calves is assumed to be an in-utero effect. The increased serum IgG at 21 d of age in calves from high CP fed dams could have been due to the increased CP intake of the dams priming endogenous protein production in their calves (Toghyani and Moharrery, 2015).

While we often focus on in-utero influences that will affect IgG absorption in neonatal calves, it is critical to examine how in-utero nutritional influences affect the lower digestive tract of the calf as well. In a study by Aragona et al. (2020), calves born to dams fed varying amounts of NA did not significantly differ in IgG absorption or AEA, despite being fed colostrum that was significantly different in IgG concentration. However, the calves born to dams fed 32 g/d NA did exhibit greater feed efficiency from wk 2-3 of life. At this time period, a calf will acquire its nutrients primarily from milk, therefore there may have been in-utero effects on enterocyte development in the calf small intestine, allowing them to absorb nutrients from milk more efficiently. Alternatively, there could have been differences in nutritional composition of the colostrum that promoted enterocyte development. Data shows the diet of the dam to impact IgG

absorption in their offspring, possibly due to altered pinocytosis or intestinal receptors, however further research is needed to explain this relationship.

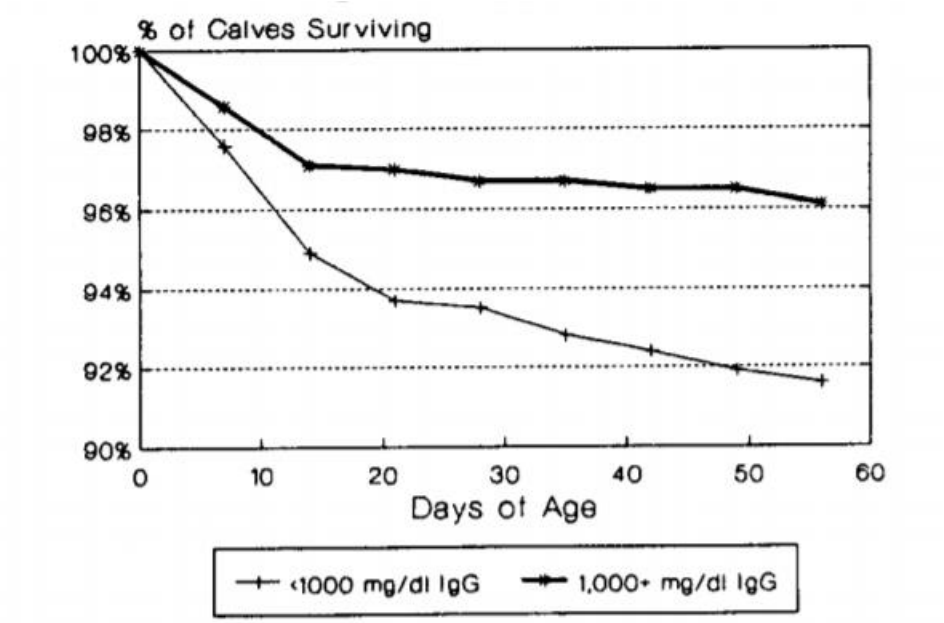

Figure 1. Survival rate of calves over time decreased when less than 1,000 mg/dL serum IgG is acquired from colostrum. (USDA , 1993).

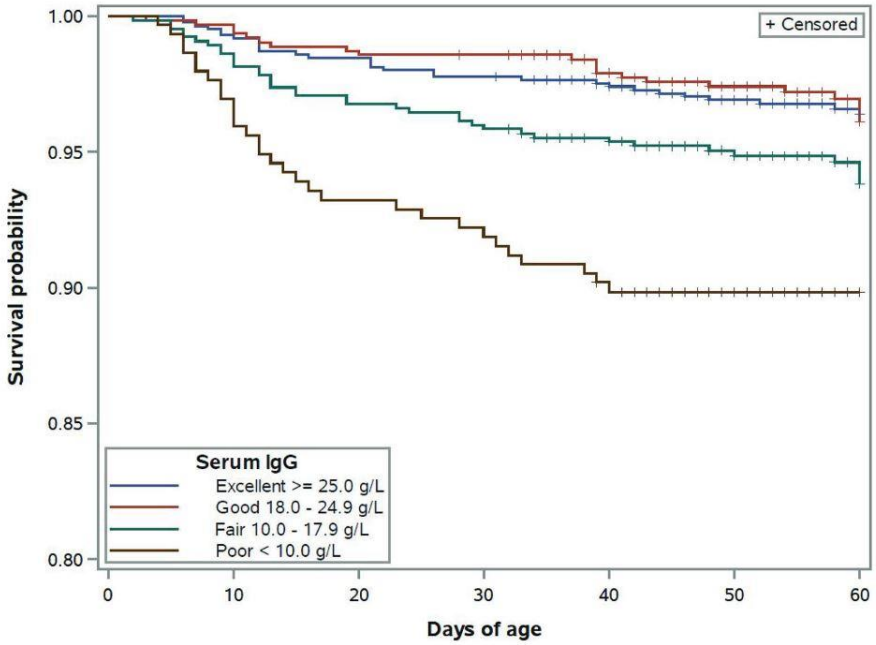

Figure 2a. From Lombard et al. (2020), adapted from Urie et al. (2018b). Survivability of calves was increased when serum IgG concentrations were greater than 18 g/L.

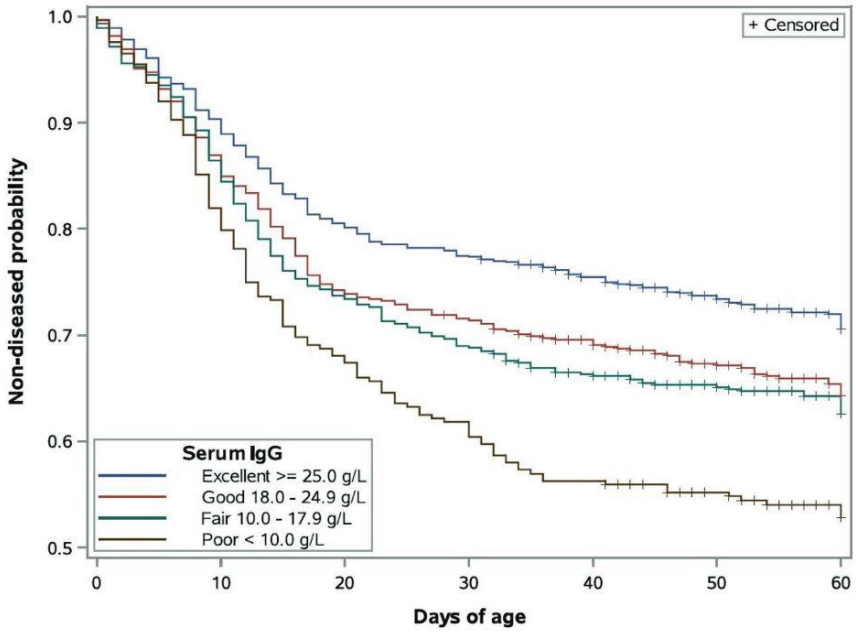

Figure 2b. From Lombard et al. (2020), reprinted from Godden et al. (2019). Probability of not acquiring disease was greatest in calves with serum IgG concentrations ≥25 g/L.

Iodine and its Role in Animal Nutrition

Dietary Iodine Requirements and Toxicity

Iodine is a necessary trace mineral in the diet of cattle that controls TH metabolism, and in turn energy metabolism. About 1.5 mg I/d is incorporated into TH formation in late gestation cows; that number increases to 4-4.5 mg I/d in lactating cows due to elevated TH production (NRC, 2001). While 80-90% of dietary I is absorbed, <20% of dietary I is used by the thyroid gland when dietary I exceeds nutritional requirements. The dietary recommendations for I are 0.33 mg/kg intake dry matter (**DM**) for dry cows and 1.5 mg/100 kg body weight in lactating cows (NRC, 2001).

Inadequate dietary I can lead to deficiency symptoms. In cows, an I deficiency can result in an enlarged thyroid gland, increased morbidity, and reduced fertility rates (NRC, 2001). Pregnant cows that are I deficient will become especially efficient in absorbing I from the blood and recycling I from TH metabolism; however, this is not enough I to support the growing fetus. Therefore, I deficiency symptoms usually manifest in the neonatal calf; I-deficient calves can be born hairless, dead, or weak, and can be aborted at any time during gestation (NRC, 2001). A dietary I intake of 50 mg/d can cause toxicity symptoms. Cows with I toxicity will exhibit excessive nasal and eye discharge, scaly coats, coughing, salivation, and reduced milk production (NRC, 2001). There is limited data to suggest the effects of I toxicity in-utero on prenatal calves.

It is estimated that forages provide only 17% of I consumed in the cow's diet, while mineral mixes are responsible for providing 83% of dietary I requirements (Borucki Castro et al., 2011). Therefore, it is difficult to provide adequate dietary I without supplementation. However,

in a study conducted in Canada, it was found that 27% of mineral mixes had I values exceeding 100,000 μg/kg, which would be considered high in I (Borucki Castro et al., 2011). It was also found that 86% of the farms on this study were supplying excess dietary I. Producers must ensure they are not overfeeding or underfeeding mineral mixes that supply I due to concerns regarding I toxicity.

Ethylenediamine dihydroiodide (**EDDI**) is an I salt used to supplement I in cattle diets that is retained in tissues longer and may be more absorbable than other I sources such as sodium or potassium iodide (Miller and Swanson, 1973). Other feed additives commonly used to supplement I include potassium iodide. A study comparing feeding EDDI with feeding potassium iodide found that the source of I in the diet did not affect thyroid hormone metabolism, or transfer of I to milk (Swanson et al., 1990). Fish and Swanson (1982) supplemented EDDI to calves, but found lower calf serum I concentrations than a study with similar feed intake where calves were supplemented with calcium iodate as an I source (Newton et al., 1974). In this case, these supplements appeared to be metabolized differently. In the study presented in Chapter II, EDDI was chosen as the inorganic I source to compare to feeding I via *Ascophyllum nodosum* (**ASCO**) meal.

Thyroid Hormone Metabolism

Dietary I is required for the production of the thyroid hormones triiodothyronine (**T_3**) and thyroxine (**T_4**). Thyroxine is converted to T_3, the active form of TH in thyroid metabolism that binds to TH receptors. Thyroid hormone production is essential for life, as TH maintain homeostasis and promote metabolism including elevating heart and respiration rates, body temperature, intake, and nutrient degradation and absorption (Saladin, 2010). Thyroid hormones

also support animal growth, as they stimulate growth hormone production from the pituitary gland.

While it is known that low I consumption of the dam can cause low TH production, or hypothyroidism, in the prenatal calf, it is less clear how excess I consumption alters cow and neonatal calf TH metabolism. An early study by Convey et al. (1978) fed supplemental I as EDDI to cows at a rate of 200 to 400 times dietary requirements for 49 wk and found no differences among TH production. Another study by Fish and Swanson (1982) fed EDDI to postweaned heifer calves at rates of 0.625, 1.25, 2.5, or 5.0 mg I/kg BW and found no differences between treatments on plasma T_4 or T_3. While a majority of data shows that feeding varying dietary I amounts to lactating cows and heifers has minimal effects on TH metabolism, there is little data on whether supplementing high I during the transition period can affect calf development in-utero. Boland et al. (2008) found that feeding excess I as calcium iodate to ewes 3 wk prior to parturition caused a reduction in total and free T_3 in the blood of their lambs, suggested a decreased rate of T_4 to T_3 conversion. More recent work feeding ewes excess I as calcium iodate (26.6 mg I/d) found excess I supplementation to decrease total (McGovern et al., 2017) and free T_3 (McGovern et al., 2016) in their lambs at 1 h postpartum. In a different study where pregnant ewes were fed excess I as calcium iodate, plasma T_3 concentrations at birth, as well as plasma T_4 concentrations at birth and 24 h increased linearly in lambs as incremental amounts of I were added to the dam's diet (Rose et al., 2007). Dietary I amounts ranged from 5.5 to 21.0 mg I/kg DM. In a study using dairy cows, boluses containing 6800 mg I, 1000 mg Se, and 1000 mg cobalt were fed 58 d before calving and it was found that TH levels of the dam's calves were unaffected, despite total and free T_3, as well as total T_4 being elevated in the dams in response to the bolus (Rose et al., 2012). These varying results suggest that TH metabolism is

complex and that more research needs to be done to determine the mechanism behind excess dietary I in cows and the subsequent effects on TH metabolism in their calves.

Iodine and Immunoglobulin G Absorption

It is of interest whether excess dietary I can affect IgG absorption in the neonatal calf, as obtaining successful passive transfer is critical for calf survival. Boland et al. (2008) found that supplementing excess I (26.6 mg I/d) as calcium iodate for 1 wk or 3 wk prepartum in ewes resulted in decreased lamb serum IgG concentrations at 24 and 72 h compared to control lambs, despite all lambs having received maternal colostrum containing adequate IgG concentration, indicating that excess dietary I prevented the lambs from absorbing colostral IgG. This is supported by lambs fed excess I having significantly lower AEA than control lambs. Other studies in ewes feeding the same amount and source of I also found 24 h serum IgG to be decreased in lambs born to ewes supplemented excess I (McGovern 2016; 2017). Data by Rose et al. (2007) in ewes found that incremental amounts of I in the dam's diet linearly decreased IgG absorption in lambs fed colostrum replacer. Excess I supplementation of 26.6 mg/d in ewes has been found to decrease gene expression of TH receptor β in the ileum of their lambs, which correlates with a decrease in free T_3 concentrations (McGovern et al., 2016; 2017). Thyroid hormones are involved in cellular differentiation as well as growth, therefore it is hypothesized that decreased expression of TH receptor β and reduced free T_3 may inhibit cellular development of the digestive tract, thus leading to less IgG absorption (Underwood and Suttle, 1999; Pàcha, 2000; McGovern et al., 2016)

While data in sheep presents a relationship between maternal I supplementation and subsequent blood TH and IgG concentrations in their lambs, data in cattle differs. When dairy

calves were fed colostrum from dams supplemented with excess dietary I, serum IgG concentrations of the calves and AEA were unaffected (Conneely et al., 2014). Similar results were seen in calves born to dams fed a bolus containing 6800 mg I 58 d before calving (Rose et al., 2012). However, Rose et al. (2012) found a positive correlation between calf 24 h serum IgG and plasma 24 h free T_3 and 1 h total T_4 concentrations. Serum IgG at 24 hours increased as free T_3 concentrations increased, while an increase in total T_4 was correlated with a decrease in serum IgG (Rose et al., 2012). It is worth noting that in this study the lack of effect on calf IgG could be due to how the bolus releases I over the period leading up to parturition. The results of Conneely et al. (2014) could have been confounded by feeding colostrum varying in nutritional composition, as well as a treatment structure where calves were fed colostrum from dams belonging to different treatment groups than the dam they were born to.

While work in ewes shows excess dietary I supplementation to the dam to suppress IgG absorption in lambs, the limited data available in dairy cows does not support this. There is the potential for differences in I metabolism between species, or that research conducted in dairy calves has various limitations that are leading to contradictory results. These limitations could include different species of iodine being used, differences in treatment structure, or potentially differences in health of the calves. It is hypothesized that TH metabolism is related to IgG absorption in the neonate, however it is unclear how dietary I, TH production, and intestinal absorption are related due to the complexity of TH metabolism.

Seaweeds

Interest in Seaweed as a Livestock Feed

Brown seaweeds have been fed in low concentrations to ruminants for decades (Makkar et al., 2016). While brown seaweeds were originally fed to provide mineral supplementation in ruminant diets, there is now an interest in feeding seaweeds to reduce methane production (Makkar et al., 2016). Specifically, red seaweeds belonging to the genus *Asparagopsis* (particularly *A. taxiformis*) have been researched for their ability to inhibit methanogenesis via impacts on methanogenic archaea in the rumen (Glasson et al., 2022). Inclusion rates of 1% or less of dietary organic matter have been shown to reduce methane production in cattle (Glasson et al., 2022). However, data shows that including *A. taxiformis* in cattle diets at rates of up to 1.0% of DM caused palatability issues and decreased dry matter intake (**DMI**), likely due to the presence of halogenated, anti-methanogenic compounds (Min et al., 2021). While *Asparagopsis spp.* has garnered interest because of its potential to reduce methane emissions, other seaweed species such as ASCO are being researched for their unique nutritional properties that may benefit the health of cattle.

Kelp meal (**KM**), produced from dried and ground seaweed, is gaining popularity as a feed supplement in agriculture. *A. nodosum* is the most prevalent species fed to livestock and is typically marketed as “kelp meal”. *A. nodosum*, also called Norwegian kelp or rockweed, is a brown seaweed native to the North Atlantic coast. With its long-life span and its ability to provide habitat for over 100 species of fish and invertebrates, it serves an important role in its ecological niche (Seeley and Schlesinger, 2012). Marketing of ASCO as a sustainable and organic product has created a global demand for this seaweed, leading to an increase in

harvesting. By removing a species vital to organisms belonging to low trophic levels, higher tropic level species could be impacted via loss of food (Seeley and Schlesinger, 2012). More research is needed to determine the effects of large-scale harvest of ASCO on marine ecosystems before it should be used by the agricultural industry on a mass scale.

The use of ASCO to feed dairy cattle is popular, particularly in the organic sector; surveys have shown that 58% of northeastern U.S. organic dairy producers feed ASCO meal as a feed supplement to cows (Antaya et al., 2015), while in Minnesota, 82.9% of organic farms fed seaweed (Sorge et al., 2016). Besides being marketed as a sustainable feed supplement, surveyed farmers state they feed ASCO to cows because it supposedly decreases incidence of pinkeye, decreases milk somatic cell count, improves body condition, and repels flies (Antaya et al., 2015). Studies have shown that ASCO does not improve milk production or components, meaning farmers may be paying for an expensive feed supplement that does not generate increased profits (Antaya et al., 2015, 2019). Per North American Kelp, a 22.68 kg bag of ASCO can cost $70.00, or $90.00 for organic ASCO. At a recommended feeding rate of 57-113 g/cow, the cost of feeding ASCO can add up quickly, and therefore must create increased profit in order to be economically feasible for farmers. In addition to its high price, palatability is another issue with feeding ASCO. When preweaned dairy calves were fed ASCO meal, it was found that calves had a preference for starter grain without ASCO, consuming 34.5% more DM from the control treatment than from the ASCO meal treatment (Erickson et al., 2012). Data where lactating cows were fed 910 g/d of marine algae (*Schizochytrium, sp.*) found a reduction in DMI of 21% (Franklin et al., 1999), however it is unclear whether ASCO has the same poor palatability in cows as it does in calves. In studies using lactating cows, incremental ASCO meal supplementation was observed to either not affect DMI (Silva et al., 2022), increase DMI on a

pasture plus concentrate diet (Antaya et al., 2019), or have trend for a quadratic relationship with DMI, with 57 and 113 g of ASCO meal resulting in greater intakes than 0 and 170 g (Antaya et al., 2015). While data suggests that feeding ASCO meal may not negatively impact intake in lactating cows, farmers should take care to ensure ASCO meal is being distributed evenly in the ration to avoid reduced DMI, and a subsequent reduction in milk yield.

Nutritional Profile of Seaweed

Seaweed is a feedstuff of interest to the dairy industry due to its unique nutritional profile. Seaweed nutritional profile is variable and is dependent on species, habitat, water conditions, time of day during harvest, and light intensity (Mišurcová, 2012). While brown algae, such as ASCO, contain less protein and more minerals than green or red algae, they do contain a great number of bioactive compounds such as antioxidants, polyunsaturated fatty acids, and vitamins (Antaya et al., 2015; Makkar et al., 2016). Additionally, ASCO is rich in phlorotannins, which are compounds that may inhibit pathogenic bacteria, as well as reduce enteric methane production and nitrogen (**N**) output into the environment (Wang et al., 2009; Zhou et al., 2018). While the reduction of methane and N output are desirable for reducing greenhouse gas (**GHG**) emissions, concerns regarding the effects of ASCO on cattle nutrition should be addressed.

Brown seaweeds typically have a mineral content of 14-35% DM, with I being one of the primary minerals present (Makkar et al., 2016). Iodine is predominately found in the environment as iodide, and has been leached from soils and into seawater due to erosion, flooding, and glaciation; seawater I concentrations are about 50 μg/L (Zimmermann, 2012). Marine plants and animals take up I from seawater and concentrate it within their systems, causing foods from marine sources to have higher I concentration than terrestrial plants and

animals. Additionally, the I present in seaweed is in its organic form as opposed to the inorganic form fed on most livestock operations (Zimmermann, 2012). While inorganic minerals are inexpensive and easy to acquire, organic sources are more bioavailable and can more efficiently supply nutrients to the animal (Evans and Critchley, 2014). Minerals in ASCO such as I are present in the form of chelates; a mineral bound to an amino acid complex which can make the mineral more bioavailable. Iodine concentration in seaweed ranges from approximately 600-1,000 mg/kg (Evans and Critchley, 2014), with the ASCO meal in our study averaging 1,066 mg/kg I. While increased bioavailability of nutrients can be desirable when they are limited in the animal's diet, health concerns become prevalent when a nutrient is present in excessive amounts.

Effects of Ascophyllum nodosum on Cow Health, Endocrine Status, and Production

There is interest in ASCO meal as a feed supplement for its potential to improve animal health. Data on the effects of ASCO meal on heat stress in lactating cows either found no alleviation of heat stress parameters such as body temperature and respiration rate in response to ASCO meal (Cvetkovic et al., 2004; Antaya et al., 2019), or only saw a reduction in rump skin temperature when 0.25% ASCO meal was added to the diet (Pompeu et al., 2011). Additionally, when blood cortisol and glucose concentrations were measured in grazing cows during the summer, there was no response to these blood metabolites in response to 113 g of ASCO meal supplementation, further indicating that heat stress was not mitigated (Antaya et al., 2019). However, when lactating cows were supplemented with 0 to 170 g ASCO meal in the winter months, serum cortisol concentrations tended to decrease linearly as greater amounts of ASCO meal were fed (Antaya et al., 2019). This suggests that ASCO meal may have helped cows

maintain their core body temperature in the cold, possibly by the microminerals in ASCO supporting increased antioxidant activity (Antaya et al., 2015). Currently, ASCO meal seems to have limited benefits in regards to mitigating heat stress in cattle, however mitigation of cold stress warrants further research.

The amount of I in ASCO meal is often in excess of the animal's nutritional requirements, which could lead to potential toxicity. A study involving feeding 113 g/d ASCO meal to grazing Jerseys found that blood concentrations of cortisol, glucose, fatty acids, and T_4 were not affected by diet (Antaya et al., 2019). Additionally, milk yield and milk component production, as well as intake of the pellet ASCO meal carrier, were not affected by ASCO meal supplementation (Antaya et al., 2019). However, serum T_3 decreased by 14% during the last period of the study, suggesting that TH metabolism may change when I is fed in excess for extended periods of time. Another study investigated feeding incremental amounts of ASCO meal (0, 57, 113, or 170 g/d) to Jerseys fed a total mixed ration (**TMR**) diet and found that feeding ANOD did not alter milk yield or components, or T_4 and T_3 (Antaya et al., 2015). Silva et al. (2022) also fed ASCO meal to lactating Jersey cows in amounts of 0, 57, 113, and 170 g/d and found a quadratic tendency for thyroid stimulating hormone (TSH) to be greatest in the 2 intermediate ASCO meal amounts, but this did not translate to a biological effect in regards to T_4 and T_3. Hong et al. (2015) fed brown seaweed by-products, rather than seaweed meal, to lactating Holstein cows and did not observe any effects on TH metabolism. While it is unclear why the high I concentration of seaweed generally fails to elicit a TH metabolism response, it could be due to the tendency of the thyroid to only use 20% of consumed I when I is present in excess (NRC, 2001). Iodine excretion (mg/d) in milk (Antaya et al., 2015; Silva et al., 2022), urine, and feces (Silva et al., 2022) has been shown to be greater than I intake from ASCO meal-

supplemented diets, with a majority of excess I being recycled and excreted via the digestive tract (Miller et al., 1975). Additionally, there is a lack of understanding on how I is absorbed from seaweed sources, as well as how I from seaweeds interact with inorganic species in the diet. The high milk I concentration presented in these studies is concerning because children are particularly sensitive to high dietary I levels and can exhibit increased thyroid gland size when excess I is consumed (Zimmermann et al., 2005).

In addition to its effects on endocrine status, there is interest in how ASCO meal affects nutrient degradation in cows. In-vitro studies have shown the phorotannins in ASCO to decrease ruminal fermentation of forages and protein degradation, likely by inhibiting the growth of cellulose-digesting ruminal bacteria (Wang et al., 2008). This could be of particular concern to farmers who utilize pasture systems, as a majority the herd's nutrient consumption would come from forage, making ASCO inclusion in the diet potentially detrimental to fiber digestibility and milk fat synthesis. However, in-vivo studies have shown incremental supplementation of 0 to 170 g of ASCO meal to linearly increase digestibility of DM, CP, and organic matter in lactating cows (Silva et al., 2022). Antaya et al. (2015) fed the same amounts of ASCO meal found acid detergent fiber (**ADF**) digestibility to respond quadratically to ASCO meal supplementation, as well as trends for quadratic responses of organic matter and NDF digestibility, with digestibility of ADF, NDF, and organic matter being greatest at a feeding level of 113 g ASCO meal. It is hypothesized that when 113 g ASCO meal were fed, there were not enough phlorotannins to inhibit cellulolytic bacteria growth as seen in Wang et al. (2008), and that the nutrients in ASCO, such as vitamin B, may have acted as a substrate for growth of cellulolytic bacteria (Antaya et al., 2015). More in-vivo research is needed to determine how different amounts of ASCO meal in the diet can affect forage degradation and its effects on cellulolytic bacteria in the rumen.

While studies in lactating cows have not shown ASCO meal supplementation to improve milk components or production (Antaya et al., 2015; 2019), some studies have shown that the phlorotannins in ASCO may improve colostrum composition. When chestnut tannins were supplemented to prepartum cows, it was observed that dietary tannins increased colostral IgG concentration (Prodanović et al., 2021). This may be due to increased amino acid flow to the small intestine and in turn, increased IgG synthesis in the mammary gland. In-vitro data has shown the presence of terrestrial tannins in rumen fluid to increase efficiency of microbial protein synthesis (^{15}N incorporation/unit short chain fatty acid production) (Makkar et al., 1995a), as well as microbial mass (Makkar et al., 1997c). While tannins decrease nutrient availability in the rumen, it is hypothesized that they increase microbial protein via a shift in nutrient partitioning, making more nutrients available to microbial synthesis and a reduction in nutrients available for short-chain fatty acid synthesis (Makkar et al., 1997c). It is possible that the phlorotannins in ASCO meal behave in a similar manner, and have the potential to improve colostrum quality and increase nutrient flow to the small intestine of cows. Islam et al. (2016) fed a fermented seaweed byproduct (**FSB**) from a brown seaweed (*Undaria pinnatifida*) to prepartum Hanwoo beef cows and examined the effects on colostrum production and calf IgG absorption. It was found that the crude fat and CP of the colostrum was significantly higher in cows fed FSB, while colostral IgG concentration increased with FSB supplementation for primiparous cows and decreased with FSB supplementation in multiparous cows. This increase in colostral fat and protein is likely due to phlorotannins reducing ruminal degradation of nutrients, allowing passage to the lower digestive tract and use in the mammary gland (Makkar 2003). Some effects could have been due to the bacteria and yeast cultures used to ferment the FSB product (Islam et al., 2016), as increased lactic and organic acids have been found to

stimulate the immune system in poultry (Friedman and Bar-shira, 2005), likely via altering the intestinal microbiome to combat pathogens (van der Wilen et al., 2000). Based on these data, there is potential for phlorotannins in ASCO meal to enhance colostrum quality.

Ascophyllum Nodosum as a Calf Feed Supplement

Some studies have investigated the effects of feeding ASCO meal to calves. In a study in which calves were fed milk supplemented with 1 of 3 types of dried seaweeds (ASCO, *Ulva lactuca,* and *Saccharina latissima*), calves fed ASCO meal had higher plasma fibrinogen and serum amyloid A concentrations on d 14 of life, as well as a tendency for increased plasma haptoglobin concentrations (Samarasinghe et al., 2021b). Plasma fibrinogen and serum amyloid A both serve as markers of the innate immune system as they promote monocyte, neutrophil, and macrophage activity, as well as increased phagocytosis efficiency (Eckersall and Bell, 2010; Ceciliani et al., 2012). Serum haptoglobin acts as an antioxidant by binding free iron in the blood that can cause cell damage, as well as sequestering iron from bacteria and acting as an antimicrobial (Ceciliani et al., 2012). Therefore, ASCO could have the potential to act as an immune system stimulant and protect calves from environmental pathogens.

When in-tact seaweeds were fed to calves, it was found that feeding ASCO in milk replacer (**MR**) caused a 13% increase in acetic acid and an 18% reduction in butyric acid in the mid-colon of calves (Samarasinghe et al., 2021a). The decrease in butyric acid in ASCO supplemented calves is concerning, as increased butyrate production is positively associated with ruminal development (Quigley et al., 1991). Currently, it is unclear whether seaweed supplementation, particularly ASCO, benefits the digestive tract of calves; more research needs to be done on how the bioactive compounds and mineral content of ASCO meal interact with

those already present in the animal's diet (Michiels et al., 2012; Samarasinghe et al., 2021c). While data shows an alteration in fermentation profile, it is unclear how this affects the function of both ruminal and post-ruminal digestive capacity.

Research regarding feeding seaweeds to prepartum cows and the effects on their calves is limited. Islam et al. (2016) fed *Undaria pinnatifida* FSB to prepartum Hanwoo beef cows and found that the caves from FSB supplemented dams had a higher weaning weight and ADG. Supplementation of FSB to primiparous dams resulted in calves with greater serum IgG levels than control animals, but this response was not seen in 2+ lactation cows (Islam et al., 2016). This increase in serum IgG in calves from FSB supplemented primiparous dams could be from consuming colostrum from that dams that had an enhanced nutritional profile. It is unclear why this response was only observed in primiparous animals. Brown seaweeds contain the compound laminarin which has been shown to stimulate the innate immune system (Volman et al., 2008), resulting in increased colostral IgG concentration in sows fed seaweed extracts from *Laminaria sp.* (Leonard et al., 2010). It is unclear if laminarin can be transferred from maternal circulation to the offspring via blood supply or lacteal secretions. Leonard et al. (2010) was not able to detect the presence of laminarin in mammary secretions, however Heim et al. (2015) found maternal laminarin supplementation of sows increase post-weaning feed efficiency and ileum villus height of their piglets.

It is important to consider the financial aspect of feeding ASCO meal to calves and whether the possible benefits are worth the cost. A study investigated feeding ASCO meal to group-fed calves in an organic system found that ADG was greater in control calves, and that calves fed 113.5 kg/d ASCO meal cost $0.44 more per kg of gain than calves fed no ASCO meal or those supplemented with 56.7 kg/d ASCO meal (Heins and Chester-Jones, 2015). Due to no

effect on ADG and greater costs, feeding ASCO meal to calves may not be economically viable. However, more research needs to be done to explore how ASCO meal affects the immune system of calves, as well as the possible financial benefits from this. While studies have explored the effects of feeding ASCO meal to lactating dairy cows and neonatal calves, there has been no research on the prepartum effects of feeding ASCO meal to transition dairy cows. Current research suggests that ASCO meal may not negatively affect lactating dairy cows and calves, but ASCO meal did not increase milk production or calf growth. However, if the high I concentration of ASCO could possibly affect colostrum production or fetal programming of the calf in-utero, this could lead to new considerations as to whether this product should be fed.

Conclusions and Proposed Study

Current literature suggests that excess I in the diet of lactating cows, either fed as an inorganic source or as an organic source from seaweeds, has little effect on the TH metabolism of the animal. Recent work involving feeding calves ASCO meal shows mixed results, with hypotheses that ASCO increases the immune status of the animals, but at a higher cost and potential decreases in ADG. However, data regarding the effects of prepartum ASCO meal supplementation on in-utero development of calves is limited. There are some data to suggest that high I supplementation in prepartum ewes decreases IgG absorption in their lambs, but these results are not reflected in the limited data available in dairy cows. There is also very limited data on the effects of high I supplementation and its effects on cow colostrum production and composition. Our study aimed to evaluate whether prepartum ASCO meal supplementation of transition dairy cows has negative impacts on the calf in-utero. We evaluated different amounts

of ASCO meal, as well as compare ASCO meal with EDDI. By evaluating colostrum composition, and calf IgG absorption, growth, intake, and TH metabolism, we aimed to provide insights regarding I and its effects on calf health and performance.

CHAPTER 2:

Feeding Different Iodine Sources to Dairy Cows: Effects on Cow Colostrum Production and Growth and Health of their Calves

INTRODUCTION

Ascophyllum nodosum (**ASCO**), a brown seaweed, is the most common seaweed species used as a feed supplement for lactating dairy cows (Makkar et al., 2016). *Ascophyllum nodosum* meal, commercialized as "kelp meal", is a feed supplement produced from dried and ground brown seaweed. A survey by Antaya et al. (2015) shows that 58% of northeastern U.S. organic dairy producers feed ASCO meal, while data from Minnesota reports that 82.9% of organic farms fed ASCO meal (Sorge et al., 2016). *Ascophyllum nodosum* is of interest due to its unique nutritional profile, including bioactive compounds such as antioxidants, polyunsaturated fatty acids (**PUFA**), and vitamins (Antaya et al., 2015; Makkar et al. 2016). Additionally, ASCO meal is rich in phlorotannins, which have been shown to make complexes with proteins in the rumen, allowing for more amino acids to reach the small intestine of the cow and be available for milk synthesis (Makkar, 2003). While seaweeds contain several beneficial nutrients, there is concern with their high uptake of iodine (**I**) from seawater (MacArtain et al., 2007); Antaya et al. (2015) reported that the I concentrations of ASCO meal averaged 820 mg/kg of dry matter (**DM**). While there is literature examining the effects of ASCO meal supplementation on lactating cow production and performance (Antaya et al. 2015; 2019; Silva et al., 2022), there is limited

research on how the I present in ASCO meal affects in-utero calf health and performance and cow colostrum production.

Colostrum is the first secretion of cattle after parturition and contains a variety of unique compounds that support the growth and health of the neonatal calf including antibodies such as immunoglobulin G (**IgG**), growth factors, and antimicrobials (Pakkanen and Aalto, 1997). It also contains nutrients critical to the newborn calf including fat, protein, carbohydrates, minerals, and vitamins (Pakkanen and Aalto, 1997). The formation of colostrum is referred to as colostrogenesis and begins approximately several weeks prior to parturition (Brandon et al., 1971). Because this timeframe corresponds to the transition period, there is interest in how the prepartum diet of the dam can alter formation of colostrum. Neonatal calves are born hypogammaglobulinemic and the absorption of IgG by the intestinal barrier ceases at approximately 24 h after birth (Stott and Fellah, 1983). Therefore, it is critical that calves are fed high-quality colostrum immediately after birth. Calves with a 24 h serum IgG concentration of 10 g/L are considered to have achieved successful "transfer of passive immunity" (**TPI**) (USDA, 1993). However, new recommendations suggest that 24 h serum IgG concentrations of ≥18 g/L are more effective at reducing calf disease and mortality (Lombard et al., 2020). Failure to promptly feed high-quality colostrum to neonatal calves can lead to disease and death of calves (USDA, 1993; Weaver et al., 2000).

There is little research on whether I present in ASCO meal can impacts colostrum production of the dam, as well as IgG absorption and thyroid hormone (**TH**) metabolism in their calves. In studies using prepartum ewes, it was found that lambs born to ewes fed excess amounts of 26.6 mg/d for 3 wk prepartum I had decreased serum IgG concentrations (Boland et al., 2008) and apparent efficiency of absorption (**AEA**) (Rose et al., 2007; Boland et al., 2008),

regardless of colostrum quality fed. Additionally, Boland et al. (2008) observed that lambs born to ewes fed excess I to have lower serum total and free triiodothyronine (**T_3**) than those born to ewes fed no I supplementation. In a study by Conneely et al. (2014), prepartum dairy cows were supplemented with 15 mg I/kg dietary DM as a top-dressed mineral powder for 7 wk, and there were no effects on calf serum IgG at 24 h or AEA. However, some calves were fed colostrum from dams belonging to different treatments than their dam. In a different study, when prepartum cows were supplemented with 6,800 mg I via a bolus 58 d prior to calving, no effects were seen on calf serum IgG or AEA (Rose et al., 2012). It is unclear why discrepancies exist between the studies executed in sheep and in cattle.

Studies feeding ASCO meal to preweaned calves found that ASCO meal increased immune markers such as serum amyloid A and plasma fibrinogen (Samarasinghe et al., 2021b, as well as increased acetate and decrease butyrate in the hindgut (Samarasinghe et al., 2021a). Feeding Hanwoo beef cows a fermented seaweed byproduct (**FSB**) (*Undaria pinnatifida*) to dams resulted in calves with greater average daily gain (**ADG**), weaning body weight **(BW**), and serum IgG concentrations on d 15 of life than those in which their mothers were not supplemented with FSB (Islam et al., 2016). Additionally, cows fed FSB had greater crude fat and crude protein (**CP**) concentrations in their colostrum. Phlorotannins in seaweeds form complexes with proteins and reduce ruminal degradation, allowing more nutrients to bypass the rumen and enter the small intestine of the cow and increasing rumen undegradable protein (**RUP**) (Makkar 2003). The increased serum IgG in the calves could have been due to consumption of nutritionally enhanced colostrum from FSB supplemented dams, or due to immune promoters such as laminarin present in seaweeds (Volman et al., 2008; Islam et al., 2016). It is still unclear how the presence of high I levels and phlorotannins in ASCO can affect

dairy cow colostrum production and calf development in-utero, as well as how feeding ASCO meal compares to inorganic I sources such as ethylenediamine hydroiodide (EDDI). It is possible the presence of phlorotannins in ASCO meal could enhance colostral protein in prepartum cows due to increased RUP and amino acid flow to the small intestine (Makkar, 2003), but based on data from sheep it has the potential to interfere with IgG absorption of calves born to ASCO meal supplemented dams (Rose et al., 2007; Boland et al., 2008).

We hypothesized that feeding ASCO meal would increase colostral nutrient concentration because of phlorotannins decreasing nutrient degradation in the rumen and improving amino acid flow to the small intestine. Additionally, we hypothesized feeding ASCO meal to prepartum dams would decrease serum IgG concentration and TH concentration in their calves via decreased gene expression of TH receptors in the small intestine. The primary objectives of the experiment were: 1) to evaluate how feeding different amounts of ASCO meal in the diet of prepartum dairy cows affects colostrum production, as well as the growth, IgG absorption, and metabolism of their calves, and 2) to determine if the same amount of I fed as either ASCO meal or EDDI can affect these parameters differently.

MATERIALS AND METHODS

All animal procedures used in this experiment were approved by the University of New Hampshire Institutional Animal Care and Use Committee (Protocol #210203). The experiment was conducted at the University of New Hampshire Fairchild Dairy Teaching and Research Center (Durham) from June 2021 to May 2022.

Part I. Prefresh Cows

Experimental Design

Forty pregnant, nonlactating, multiparous Holstein cows were blocked by lactation number and expected calving date, and assigned to 1 of 4 dietary treatments in a manner that made average lactation number among treatments as even as possible. Lactation number averaged (mean ± standard deviation) 3.2 ± 1.1. The 4 treatments consisted of: (1) EDDI added to meet NRC (2001) recommended concentration of I (0.5 mg /kg of DMI; control diet = **CON**), CON plus 57 g of ASCO meal (low seaweed supplementation diet = **LO**), CON plus 113 g of ASCO meal (high seaweed supplementation diet = **HI**), and CON plus EDDI added to match the amount of I supplied by 113 g of ASCO meal (i.e., 124.8 mg of I; **EDDI** diet). *Ascophyllum nodosum* meal was purchased from SOURCE Micronutrients (Branford, CT). Amounts of ASCO meal were selected based upon manufacturer recommendations and previous research done in our laboratory (Antaya et al., 2015, 2019; Silva et al., 2022). *Ascophyllum nodosum* meal or EDDI was added in addition to the CON amount of I in order to reflect farm practices of feeding ASCO meal in addition to I mineral powders. Iodine sources were top-dressed to a basal diet fed to all cows that contained no added iodine and manually mixed within the top layer of the TMR to ensure uniform distribution of I sources. Cows were enrolled on the study 28 d (±2 d) prior to expected calving date and fed the experimental diets during this period.

Animal Management

Cows were housed in a bedded-pack barn with dried pine shavings as bedding. The feed bunk was fitted with a Calan Broadbent Feeding System (American Calan) to individualize intake. Cows were given access to the Calan doors 7 d prior to being enrolled in the study to get

used to the system. The farm dry cow diet containing primarily corn silage and a prefresh grain mix was fed to cows during the Calan door training period, and then shifted to the experimental diets on d 1 of enrollment. A week prior to expected calving date, cows were moved to individual maternity stalls equipped with feed tubs to monitoring for calving from approximately 2100 h to 0400 h for ±7 d prior to calving, cows were fitted with calving sensors (MooCall Inc.) to the tailhead to alert researchers of calving.

Colostrum Sampling and Analyses

Upon calving, all cows were injected intramuscularly with 5 cc oxytocin for aiding with milk ejection and expulsion of placenta. Cows were milked for colostrum within 1 h of calving using a portable bucket milker (DeLaval). Teats were fore stripped and sprayed with DeLaval Prima™ teat disinfectant (DeLaval) prior to milking, as well as dipped in WestAgro® Blockade™ iodine solution (WestAgro®) after milking was completed. After colostrum was harvested, it was weighed using a hanging scale (RoMech) with a 300 kg weight capacity, and the weight was recorded. Two 50-mL vials were filled with colostrum and stored at -20°C (vial 1) or 4°C (vial 2) both without preservative. Samples stored at -20°C were shipped to The Saskatoon Colostrum Co. Ltd. (Saskatoon, SK, Canada) for IgG concentration analysis using radial immunodiffusion assay, as well as to Michigan State University Veterinary Diagnostic Laboratory (Lansing, MI) for I analysis via inductively coupled plasma mass spectroscopy. Samples stored at 4°C were shipped for analyses of fat (method 989.05; AOAC International, 2019), total protein and true protein (method 991.20/991.22; AOAC International, 2019), and total solids (method 990.20; AOAC International, 2019) concentrations within 3 d of sampling

(Dairy One Check Mark Laboratory, Ithaca, NY). Colostrum yield was utilized to calculate fat, true protein, I, and IgG yield.

Part II. Calves

Experimental Design

Forty-one Holstein calves were obtained from the cows used in this study, including 18 heifers and 23 bulls, with 2 of these calves being freemartin twins. Calves were assigned the same treatment group and block as their mothers. Calves were not directly fed the dietary treatments and were assigned to the same treatments received by their respective mothers. Calves were enrolled in the study from birth and stayed until d 56 of age.

Animal Management

At birth, calves were removed from the dam before nursing and housed in 1 × 2.15 m individual pens bedded with dried pine shavings. Calf was housed in an indoor, naturally ventilated calf room. Within 2 h of birth, calves were fed colostrum replacer (**CR**) containing a dose of 200 g of IgG (3 L) (The Saskatoon Colostrum Co. Ltd.), followed by another feeding of CR containing 100 g IgG (2.5 L) 6 h after birth, for a total of 300 g IgG. Calves were offered colostrum replacer from a nipple bottle and if any amount was refused, the remaining volume was fed via esophageal tube. Navels were dipped with a navel-dip solution (Vetericyn® Super 7+) to prevent infection. After feeding CR, calves were administered Tri-Shield First Defense (ImmuCell Co.) orally to protect calves against *Escherichia coli*, bovine coronavirus, and bovine rotavirus. Pens were cleaned and bedded twice daily. Calves were dehorned after d 7 of age via cauterization after lidocaine administration. Ear tags were implanted after 4 wk of age.

Over the course of the study, 10 calves suffered from scours and were treated following standard operation procedures adopted at the Fairchild Dairy Teaching and Research Center. Calves suffering from diarrhea and dehydration were offered DIAQUE electrolyte solution (Boehringer Ingelheim Animal Health USA Inc.) in the water or tube-fed at a dose of 50 g per 1.89 L water for mild cases, and 100 g per 1.89 L water for severe cases. In more severe scours cases, a 60 mL tube of First Arrival® probiotic paste (DBC Ag Products) was fed for 1-2 d. One calf was born with possible polioencephalomalacia and was treated with 2 mL of vitamin B_1 on d 2 of life, followed by 1mL/d for 3 d. One calf presented abomasal bloat and was treated with approximately 177 mL of a bloat release (AgriLabs).

Feeding, Sampling, and Analysis of feeds

Nurture® professional milk replacer (**MR**) (Provimi®) containing 24% CP and 17% fat was fed twice daily at 0500 h and 1600 h until d 49 of age, and then once daily at 0500 h from d 50 to d 56 to facilitate weaning. It was prepared by mixing 336 g of powder in 2.84 L of warm water, amounting to about 3 L per feeding. Milk replacer was medicated with 0.11 g/kg Lasalocid and 0.02 g/kg Diflubenzuron for deterrent of flies in feces. The amount of MR fed was the same for all calves and remained consistent throughout the study. Refusals, if present, were measured at both feedings.

Calves were fed a coarse starter (HerdFirst® Starter, Cargill, Minneapolis, MN) containing protein pellets, oats, and whole corn. Starter was medicated with 0.06 g/kg Monensin and 0.01 g/kg Diflubenzuron. Starter fed and refused was measured each AM feeding. Amount of starter fed was based on refusals for the previous day, allowing for an expected 91 g of orts. If starter was consumed by PM feeding, additional starter was measured and offered. Each AM

feeding, 5.7 L of water was offered, with refusals being measured the next AM. Additional water was offered if it was consumed or not clean by the PM feeding. Water and starter were both offered starting at 24 h of life.

Starter orts were collected daily and frozen at -20°C for DM analysis. Starter and MR samples were collected from each bag and frozen at -20°C for nutrient analysis. Starter and MR intakes were used to calculate dry matter intake (**DMI**) as well as feed efficiency (average daily gain (**ADG**)/DMI). Dry matter of orts, starter samples, and MR samples were determined by thawing samples for 4-8 h and drying in paper bags for 48 h at 55°C in a forced hot air convection oven (Binder, Bohemia, NY).

Body Weight, Growth, and Health Measurements

Calves were weighed at birth and then once per week until 8 wk of age using a digital scale (A and A Scales LLC). Body weights were used to calculate ADG. Body measurements were taken once per week until 8 wk of age and included hip width, body length, heart girth, hip height, and withers height. Hip width, body length, and heart girth were taken with a weigh tape (Nasco® Education) while hip height and withers height were taken using a sliding scale height stick with a bubble level (Nasco® Education). Health and fecal scores were also taken once weekly until 8 wk of age using the University of Wisconsin (Madison, WI) calf health scoring system. Weekly sampling date was based on calf birth date as follows. Calves born after Thursday 1600 h and before Monday 0700 h were weighed and sampled on Tuesdays at 0700 h, while calves born after Monday 0700 h and before Thursday 1600 h were weighed and sampled on Fridays at 1630 h.

Blood Sampling and Analyses

Blood samples were obtained via jugular venipuncture. Calves were sampled for blood on d 0 of life (day of birth) within 1 h of parturition and before colostrum feeding (0 h), as well as 24 h after birth (24 h). At 0 h and 24 h, 2 10 mL vacutainer tubes of blood were taken, with the first containing no additive for whole blood collection and the second containing EDTA for plasma collection. Blood was also sampled once weekly until 8 wk of age using the same schedule as BW and skeletal measurements. For weekly sampling, 2 10-mL vacutainer tubes containing EDTA were obtained from each calf, and 1 mL of whole blood was collected for BHB analysis. Blood was centrifuged at 1,278 × *g* at 4°C for 20 min. Serum was frozen for IgG analysis, while plasma was frozen for TH analysis including total T_3 and total T_4, as well as free T_3 and free T_4 using ELISA commercial kits (Monobind Inc.). Whole blood was analyzed immediately for BHB in duplicate using the Nova Vet handheld meter (Nova Biomedical). Serum samples were sent to The Saskatoon Colostrum Co. Ltd. for IgG analysis using radial immunodiffusion assay. Apparent efficiency of absorption of IgG was calculated using the following equation by Quigley et al. (1998):

AEA = [(serum IgG at 24 h (g/L) × BW (kg) x 0.09) / colostral IgG intake (g)] × 100

In addition to initial and weekly blood sampling, a xylose challenge was run on d 5 of life to be used as a proxy for intestinal development as D-xylose has less affinity for glucose transporters than glucose and thus will be more selectively absorbed (Scharrer and Grenacher, 2000). Calves were fed 0.5 g/kg BW of 99+% D-xylose (Acros Organics) mixed into the morning milk feeding on d 5 of life. Grain was removed and calves were not fed milk for 12 h. Bloods samples were taken at 0 h before xylose feeding, then at 2, 4, 6, 8, and 12 h thereafter to obtain an absorption curve, and processed for plasma Samples were centrifuged and stored as

reported previously for TH. Samples were analyzed for plasma glucose using a commercial kit (Fujifilm Wako Chemicals). Samples were also analyzed for plasma xylose following protocol by Merritt and Duelly (1983), with some modifications. Specifically, samples (300 µL) were transferred to a 96-well plate after cooling down and vortexed, and finally read in an Epoch microplate spectrophotometer with Gen5™ analysis software (Agilent) instead of using cuvettes as reported in the original procedure of Merrit and Duelly (1983).

Statistical Analyses

Preplanned, nonorthogonal contrasts were used to determine if there were linear and quadratic effects of incremental amounts of ASCO meal (0 g/d = CON; 57 g/d = LO; 113 g/d = HI), as well as if there were any differences between feeding the same amount of I from EEDI versus the greatest level of ASCO meal (EDDI vs. HI). Data were analyzed according to a randomized complete block design using, the MIXED procedure of SAS 9.4 (SAS Institute Inc.). Normality was tested using the Shapiro-Wilk test. MIXED was used analyze data following a normal distribution or data that could be transformed to fit normality. If the data did not fit a normal distribution and could not be transformed to fit a normal distribution, the GLIMMIX procedure was used. The GLIMMIX procedure provides a better-fitting model for data that does not fit a normal distribution (SAS Institute, Inc.). Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$. Outliers within the data were declared at ± 3.0 standard deviations from the mean and subsequently removed from the dataset.

Cow data. Colostrum yield, and concentrations and yields of fat, total protein, true protein, total solids, I, and IgG were analyzed according to the following model:

$$Y_{ij} = \mu + B_i + TRT_j + \beta X_{ij} + e_{ij},$$

where Y_{ij} = the dependent variable, μ = the overall mean, B_i = the random effect of *i*th block (*i* = 1,…,10), TRT_j = the fixed effect of the *j*th treatment level (*j* = 1, 2, 3, or 4), β = the regression (covariate coefficient), X_{ij} = the covariate measurement, and e_{ij} = the residual error. In this model, the random effect of cow within block subclass was used as the error term for the effect of treatment. Parity of the dam was used as the covariate in this model. If $P \geq 0.25$, the covariate term was removed from the model. Degrees of freedom were calculated using the Kenward-Roger approximation option for the MIXED and GLIMMIX procedures. Iodine concentration and yield did not follow a normal distribution and were analyzed using the GLIMMIX procedure, while all other parameters were analyzed using the MIXED procedure.

Calf data. Initial, final, and overall gain of calf BW and calf skeletal measurements (hip height, withers height, heart girth, body length, and hip width), 0 and 24 h plasma TH concentration and IgG, IgG AEA, initial and final BHB, and plasma total and free T_4/T_3 ratios were analyzed according to the following model:

$$Y_{ij} = \mu + B_i + TRT_j + \beta X_{ij} + e_{ij},$$

where Y_{ij} = the dependent variable, μ = the overall mean, B_i = the random effect of *i*th block (*i* = 1,…,10), TRT_j = the fixed effect of the *j*th treatment level of the dam (*j* = 1, 2, 3, or 4), β = the regression (covariate coefficient), X_{ij} = the covariate measurement, and e_{ij} = the residual error. In this model, the random effect of calf within block subclass was used as the error term for the effect of treatment. Parity of the dam and calf sex were used as covariates in this model. If $P \geq 0.25$, the covariate term was removed from the model. Calf BW was not used as a covariate in this experiment because of its potential to be an effect of treatment. Degrees of freedom were calculated using the Kenward-Roger approximation option for the MIXED and GLIMMIX procedures. Final BW, initial, final, and overall hip width gain, 0 h plasma total T_4, 0 h plasma

total T_3, 0 h plasma free T_3, 0 h serum IgG, and initial and final plasma BHB concentrations were all analyzed using the GLIMMIX procedure as they did not fit a normal distribution. Remaining variables fit a normal distribution and therefore were analyzed using the MIXED procedure.

Calf intakes, ADG, feed efficiency (**FE)** (ADG/DMI), weekly BW, weekly skeletal measurements (hip height, withers height, heart girth, body length, and hip width), and weekly BHB were analyzed as repeated measures according to the following model:

$$Y_{ijkl} = \mu + B_i + TRT_j + W_k + \beta X_{ij} + TRT \times W_{jk} + e_{ijkl}$$

where Y_{ijkl} = the dependent variable, μ = the overall mean, B_i = the random effect of *i*th block (*i* = 1,…,10), TRT_j = the fixed effect of the *j*th treatment level of the dam (*j* = 1, 2, 3, or 4), W_k = the fixed effect of the *k*th week relative to birth, β = the regression (covariate coefficient), X_{ij} = the covariate measurement, $TRT \times W_{jk}$ = the fixed interaction between the *j*th treatment level and the *k*th week, and e_{ijkl} = the residual error. In this model, the random effect of calf within block subclass was used as the error term for the effect of treatment. Parity of the dam and calf sex were used as covariates in this model. If $P \geq 0.25$, the covariate term was removed from the model. Calf BW was not used as a covariate in this experiment because of its potential to be an effect of treatment. Degrees of freedom were calculated using the Kenward-Roger approximation option for the MIXED and GLIMMIX procedures. Milk replacer intake and ADG were modeled using a variance components structure. Starter intake was modeled using an unstructured model. Body weight was modeled using a Toeplitz structure. Withers height, hip height, body length, heart girth, hip width, DMI, FE, and BHB were modeled using an autoregressive-1 structure. The model structure was selected based on which covariance matrix produced the lowest Bayesian information criterion. Starter intake, milk replacer intake, total DMI, weekly BW, withers height,

heartgirth, body length, and hip width, as well as ADG, FE, and weekly plasma BHB concentration were all analyzed using GLIMMIX as they did not fit a normal distribution. Remaining variables fit a normal distribution and therefore were analyzed using the MIXED procedure.

Plasma TH concentration over time and plasma TH ratios over time were analyzed as repeated measures according to the following model:

$$Y_{ijkl} = \mu + B_i + TRT_j + D_k + \beta X_{ij} + TRT \times D_{jk} + e_{ijkl}$$

where Yi_{klj} = the dependent variable, μ = the overall mean, B_i = the random effect of *i*th block (*i* = 1,…,10), TRT_j = the fixed effect of the *j*th treatment level of the dam (*j* = 1, 2, 3, or 4), D_k = the fixed effect of the *k*th day relative to birth (*k* = 0, 1, 14, 28, 56), β = the regression (covariate coefficient), X_{ij} = the covariate measurement, $TRT \times D_{jk}$ = the fixed interaction between the *j*th treatment level and the *k*th day, and e_{ijkl} = the residual error. In this model, the random effect of calf within block subclass was used as the error term for the effect of treatment. Parity of the dam and calf sex were used as covariates in this model. If $P \geq 0.25$, the covariate was removed from the model. Calf BW was not used as a covariate in this experiment because of its potential to be affected by treatment. Degrees of freedom were calculated using the Kenward-Roger approximation option for the MIXED and GLIMMIX procedures. Total plasma T_4 and T_3 concentrations were modeled using an unstructured structure. Plasma free T_4 concentration, total plasma T_4/T_3 ratio, and plasma free T_4/T_3 ratio were modeled using a variance components structure. Plasma free T_3 concentration was modeled using an autoregressive-1 structure. The model structure was selected based on which covariance matrix produced the lowest Bayesian information criterion. All plasma TH concentrations over time and plasma TH ratios over time were analyzed using the GLIMMIX procedure as they did not fit a normal distribution.

Blood glucose and xylose concentrations on d 5 of life were analyzed as repeated measures according to the following model:

$$Y_{ijkl} = \mu + Bi + TRT_j + Hk + \beta X_{ij} + TRT \times H_{jk} + e_{ijkl}$$

where Y_{ijkl} = the dependent variable, μ = the overall mean, B_i = the random effect of *i*th block (*i* = 1,…,10), TRT_j = the fixed effect of the *j*th treatment level of the dam (*j* = 1, 2, 3, or 4), H_k = the fixed effect of the *k*th hour relative to xylose feeding on d 5 of life (*k* = 0, 2, 4, 6, 8, 12), β = the regression (covariate coefficient), X_{ij} = the covariate measurement, $TRT \times H_{jk}$ = the fixed interaction between the *j*th treatment level and the *k*th hour, and e_{ijkl} = the residual error. In this model, the random effect of calf within block subclass was used as the error term for the effect of treatment. Parity of the dam and calf sex were used as covariates in this model. If $P \geq 0.25$, the covariate was removed from the model. Calf BW was not used as a covariate in this experiment because of its potential to be an effect of treatment. Degrees of freedom were calculated using the Kenward-Roger approximation option for the MIXED and GLIMMIX procedures. Plasma xylose concentration was modeled using a Toeplitz structure, while plasma glucose concentration was modeled using a compound symmetry structure. The model structure was selected based on which structure produced the lowest Bayesian information criterion. Plasma glucose concentration fit normality and was analyzed using the MIXED procedure, while plasma xylose concentration was analyzed using the GLIMMIX procedure as it did not fit a normal distribution.

RESULTS AND DISCUSSION

Chemical Composition of Milk Replacer and Starter Grain

The nutritional compositions of the MR and starter used in this study are presented in Tables 1 and 2, respectively. Nutrient variation in the feeds is due to multiple batches of MR and starter grain used over the 11-month period that it took to complete the study.

Cow Colostrum Yield and Composition

Treatments had no effect on colostral and fat yield, as well as concentrations and yields of total and true protein, total solids, I, and IgG (Table 3). Only 1 cow on study produced colostrum below the threshold of >50 g/L IgG (Beam et al., 2009). Colostral fat concentration was 2.5 percentage units greater (P = 0.04) in HI compared with feeding the same amount of I via EDDI (Table 3). Additionally, colostral fat concentration tended (P = 0.07) to increase linearly with addition of incremental amounts of ASCO meal. These results are in partial agreement with Islam et al. (2016) who reported an increase in colostral fat concentration when feeding fermented brown seaweed byproducts (*Undaria pinnatifida*) to Hanwoo beef cows. The increase in fat percentage found in this study and the study of Islam et al. (2016) in response to feeding cows seaweed could have been due to the presence of phlorotannins in seaweed. While concentration and bioactivity of phlorotannins in ASCO tends to greatest among brown seaweed species, analyses have shown *Undaria pinnatifida* to also have high phlorotannin concentrations, differing in total phenolic content from ASCO by about 40 mg gallic acid equivalent/g dry weight extract (Shen et al., 2021). Terrestrial plant tannins have been found to decrease nutrient availability in the rumen by making complexes with proteins, thus allowing AA to be absorbed

in the small intestine and be utilized by the host ruminant rather than ruminal microbes (Makkar, 2003). Therefore, phlorotannins may function in a similar manner as they are structurally similar to terrestrial tannins, albeit less structurally complex (Wang et al., 2008). However, in-vitro data observed the presence of ASCO inhibited aNDF degradation of mixed forage, which in vivo, would lead to a decrease in production of acetate, which serves as a precursor to milk fat synthesis (Wang et al., 2008). However, Antaya et al. (2015) found a quadratic response of ADF digestibility when incremental amounts of ASCO meal (0 to 170 g) were fed to lactating cows, possibly due to the presence of vitamin B in ASCO promoting growth of cellulolytic bacteria in 57-113 g ASCO meal. While certain levels of ASCO meal may inhibit growth of cellulolytic bacteria due to high amounts of phlorotannins, it is possible that ASCO may promote cellulolytic bacteria growth, and subsequently colostral fat synthesis, when moderate amounts are fed. In our study, it is conceivable that phlorotannin intake may have allowed for more nutrients to be utilized in colostrogenesis, and subsequently to colostral fat production, although no differences were found in fat yield, possibly due to a large variation.

It is hypothesized that the phlorotannins in the fermented seaweed byproduct fed by Islam et al. (2016) also increased colostral crude protein concentration in addition to crude fat concentration. However, in our study, colostral protein was unaffected by treatment. When chestnut tannins were fed to dairy cows, an increase in colostral IgG concentration and a tendency for increased protein percentage in response to tannins in the diet was observed (Prodanović et al., 2021). Terrestrial tannins, and possibly phlorotannins, have been shown to increase rumen undegradable protein as well as microbial protein synthesis (Makkar, 2003), which could make more amino acid precursors available for colostral protein synthesis (Huhtanen et al., 2010). Previous work involving feeding brown seaweed extract to prepartum

sows also found supplementation of seaweed products to increase colostral IgG (Leonard et al., 2010). The reason for the discrepancies between our results and those of previous studies may be because we fed ASCO meal, while other studies either fed a fermented seaweed product (Islam et al., 2016) or a different species of brown seaweed such as *Laminaria spp*, which contains high amounts of the compound laminarin. (Leonard et al., 2010). Laminarin is a β-(1-3)-glucan found in brown seaweeds that is known to stimulate the nonspecific immune system (Read et al., 1996) and may also stimulate IgG production in the pregnant cow (Leonard et al., 2010). The species *Laminaria spp.* is known to contain a greater amount of laminarin than ASCO and may be more effective as an immune system stimulant (Kadam et al., 2015), which may be why we did not see increases in colostral IgG in our study. Additionally, the fermented seaweed product used by Islam et al. (2016) contained probiotics, which may have stimulated the immune system and IgG production of the dams further due to increased concentrations of lactic and organic acids in fermented feeds, which have been shown to increase immune function (Friedman and Bar-shira, 2005). Further investigation of feeding seaweed as a fermented product rather than as a dried meal is warranted. Alternatively, our colostrum measurements showed a relatively high variation which may have contributed to the lack of differences among treatments. Supplementation with ASCO meal may have benefits on colostrum composition, but further research needs to be done to gain insights on the mechanisms that ASCO phlorotannins and bioactive compounds affect colostrogenesis and nutrient partitioning in the rumen.

Colostral I concentration and yield were not affected ASCO meal supplementation in the current study (Table 3). Milk I concentration has been shown to increase linearly as ASCO meal was added to the diet (Antaya et al., 2015; 2019; Silva et al., 2022). We observed high variation in colostral I concentration measurements in our study which may have contributed to lack of

differences among treatments. Additionally, milk I results from the studies cited above were from samples pooled over time, while colostrum I was a single sample, further contributing to I variation. It should be noted that the mean colostral I concentration from LO and HI treatments observed herein (i.e., 240.60 and 235.30 ng/L, respectively) was lower than the milk I concentration of the control diet (0 g/d ASCO meal) reported by Silva et al. (2022), suggesting differences on how I is transferred to colostrum or milk. While mechanisms of micromineral transfer to colostrum are still unclear, it is possible that I passes into colostrum at a lesser rate than milk due to increased nutrient requirements of the developing fetus in the last 2 to 4 wk prior to parturition (Quigley and Drewry, 1998).

Intakes and Calf Growth Parameters

One cow on the EDDI treatment had twins, resulting in 41 calves on study; 23 bulls and 18 heifers were obtained. One set of twins, consisting of a bull and a freemartin heifer, was obtained from the EDDI treatment group. A total of 31 calves were tube-fed some portion of their first colostrum feeding: 6 CON, 6 LO, 8 HI, and 11 EDDI calves. A total of 39 calves were tube-fed some portion of their second colostrum feeding: 9 CON, 9 LO, 10 HI, and 11 EDDI calves. Table 4 shows the number of calves treated for illness, as well as the mode of treatment.

Calf intake and growth parameters are presented in Table 5. There were no differences among treatments for starter, MR, and total DMI. Similarly, ADG, feed efficiency, heartgirth, body length, and hip width were not different across treatments. Initial BW was lower in EDDI (-2.5 kg) compared with HI calves ($P = 0.02$), while there was linear tendency ($P = 0.10$) for calf BW overall gain to decrease with the addition of ASCO meal to the dams' diet (Table 5). Hip height over time ($P = 0.09$) and final withers height ($P = 0.06$) tended to be lower in EDDI than

HI calves, while final hip height (P = 0.03) and withers height over time (P = 0.05) decreased in EDDI versus HI calves. These results suggest that feeding I as an organic source such as ASCO meal as opposed to an inorganic source like EDDI led to smaller calves at birth, as well as reduced skeletal growth (hip and withers height). When fermented seaweed products from *Undaria pinnatifida* were fed to prepartum beef cows, calves born to seaweed supplemented dams had greater weaning weights, but birthweights were not different between treatments (Islam et al., 2016). However, the differences in weaning weights in Islam et al. (2016) study may have been to the suckling calves consuming maternal colostrum with improved nutrient concentration due to a potential treatment effect. Calves in our study were all fed the same colostrum replacer, and all had similar intake, therefore, birth BW and vertical skeletal growth effects seen herein are likely in-utero effects of ASCO meal versus EDDI supplementation. The tendency (P = 0.10 for incremental amounts of ASCO meal in the prepartum diet to reduce overall calf BW gain (Table 5) may be related to changes in the concentration of blood metabolites discussed in detail below.

Calf Blood Metabolites

Plasma TH and serum IgG concentrations are presented in Table 6. There were no treatment differences in the plasma concentrations of total and free T_3, and free T_4, or free T_4/T_3 ratio. Similarly, serum concentration of IgG at 0 and 24 h did not differ across treatments. Total T_4 over time exhibited a positive quadratic response (P = 0.02) with ASCO meal supplementation to the prepartum diet, with a nadir occurring with the LO group. Total T_4/T_3 ratio over time also exhibited a positive, quadratic tendency (P = 0.06) with a nadir at the LO group (Table 6). A greater T_4/T_3 ratio can be indicative of reduced T_4 to T_3 conversion (Boland

et al., 2008), however total and free plasma T_3 concentrations were unaffected by treatments, therefore the effect was likely driven by the quadratic response of total T_4. However, all other TH were unaffected by treatments. Previous data in lactating cows and heifers observed that when excess dietary concentrations were supplemented as EDDI (up to 200 mg/kg I in cows and up to 174 mg/kg in heifers), that blood TH production was unaffected (Convey et al., 1978; Fish and Swanson, 1982). In prepartum ewes, excess I supplementation of 26.6 mg/d of calcium iodate resulted in reduced total (Boland et al., McGovern et al., 2017) and free (Boland et al., McGovern et al., 2016) plasma and serum T_3 concentrations in their lambs. In studies where ASCO meal were fed to lactating cows, blood TH concentrations were generally unaffected (Antaya et al., 2015; Hong et al., 2015; Silva et al., 2022). A decrease in serum T_3 observed only when grazing cows were fed 113 g/d ASCO meal for 84 d, suggesting that ASCO meal may only affect TH production if fed for a long period of time (Antaya et al., 2019. However, studies evaluating the effects of prepartum I supplementation on TH metabolism of calves are limited. When prepartum dairy cows were fed rumen boluses containing 6800 mg of I, the dams exhibited elevated free and total plasma T_3 as well as total plasma T_4 concentrations, but their calves' TH levels were unaffected (Rose et al., 2012). It is currently unclear how the bioactive compounds and minerals in ASCO interact with other minerals in the diet (Michiels et al., 2012; Samarasinghe et al., 2021c), which could have been why most TH did not response to the treatments in our study.

It is difficult to explain why we saw a quadratic relationship with calf T_4, with CON and HI showing greater concentrations of this hormone than LO. Data in sheep observed increases in lamb 0 h plasma T_3 and T_4, and 24 h plasma T_4 concentrations when incremental amounts of calcium iodate ranging from 5.5 to 21.0 mg/lg DM were fed to the dams (Rose et al., 2007).

However, in our study we observed a nadir in calf plasma T_4 concentrations with the LO group, which had less prepartum I than HI. Cows tend to use less I for TH synthesis when I is fed in excess (NRC, 2001), therefore, we hypothesize that more I was being allocated towards fetal blood supply in the HI than the LO group, therefore priming the neonatal calf for greater T_4 synthesis in the first 8 wk of life. However, both CON and HI resulted in greater plasma T_4 concentrations than LO. It is possible that becuase the CON treatment provided the ideal concentration of prepartum I to the fetus, the CON calves were more efficient at T_4 synthesis than LO, making them comparable to HI calves. Additionally, more bioactive compounds could have transferred from maternal to fetal blood supply in HI than LO calves, resulting in increased TH synthesis, however more research is needed to support this hypothesis. Free T_4 is the portion of T_4 not bound by binding proteins, and is therefore metabolically available for conversion to total T_3. Because total and free T_3 were unaffected by treatment, it appears availability of free T_4 for conversion was unaffected as well, despite total T_4 showing a quadratic response. Therefore, it is unclear if the quadratic response of calf plasma T_4 concentrations to increasing maternal ASCO meal supplementation translated into any biological effects.

All calves on study had a 24 h serum IgG >10 g/L, the minimum for acquiring passive transfer. Immunoglobulin G AEA tended ($P = 0.06$) to be greater in HI compared with EDDI calves (Table 6). Serum IgG concentrations at 0h and 24h were similar among treatments, however AEA tended ($P = 0.06$) to be greater in HI than in EDDI calves (Table 7). The lack of serum IgG response with increasing I levels via ASCO meal in the diet is consistent with Conneely et al. (2014), who did not find differences in calf serum IgG when various levels of I were supplemented to the dam's diets ranging from 0.33 to 15 mg I/kg DM. However, this conflicts with studies in sheep where increasing I supplementation to ewes led to a decrease in

IgG absorption in their lambs (Boland et al., 2005; Rose et al., 2007; Boland et al., 2008). A similar study using beef cows found that supplementation of fermented seaweed byproducts, which are high in I, to pregnant dams caused their suckling calves to have higher serum IgG than control calves on d 15 of life (Islam et al., 2016). We may not have seen changes in 24 h serum IgG in our study because 300 g of IgG was fed to each calf, which is more than the 100-200 g IgG typically fed in colostrum replacer to obtain passive transfer. All treatment groups in this study also had relatively high AEA. It is hypothesized that excess maternal I supplementation may not affect absorption of IgG in the progeny as adversely when a high amount of IgG is fed to the neonate (Conneely et al., 2014).

While amount of I in the prepartum diet did not affect IgG absorption of the calves in our study, it seems that the source of I did cause an effect. Brown seaweeds, including ASCO, contain the compound laminarin which is known to stimulate the host immune response via β-glucans (Volman et al., 2008). Therefore, it is possible that the bioactive compounds in ASCO meal were able to stimulate IgG absorption in HI compared with EDDI calves. Decreased AEA of the EDDI calves may be an explanation for the tendencies seen for reduced hip and withers height growth seen relative to HI calves. It has been observed that when IgG absorption from colostrum is inhibited, calves will exhibit a reduction in BW gain (Ferdowsi Nia et al., 2009). Additionally, Quigley et al. (2001) found that calves with higher 24 h IgG concentrations had greater BW gain over 60 d. Therefore, calves with lower serum IgG will have to dedicate more nutrients to establishing their immune systems and therefore have less nutrients available for skeletal growth, which may have been why the EDDI calves on our study had both lower AEA and growth rates (Ferdowsi Nia et al., 2009). It is possible that the bioactive compounds present in ASCO meal helped with IgG absorption and development of the immune system of the HI

calves, however more research is needed to determine how effectively these bioactive compounds transfer to fetal blood supply.

Calf BHB is presented in Table 7. Plasma BHB over time and final plasma BHB exhibited a positive quadratic response ($P = 0.02$ and, $P = 0.05$, respectively) in response to ASCO meal supplementation, with a nadir occurring at the LO treatment (Table 7). Plasma BHB can be used as a proxy for starter intake, and subsequently BHB production in the rumen (Quigley et al., 1991). As the developing rumen relies less on glucose, it will start to synthesize BHB, which is indicative of a more developed rumen (Quigley et al., 1991). Starter intakes in our study were not different between treatments, therefore supplementation of incremental amounts of ASCO meal may have altered ruminal development. Similar to our hypothesis for plasma T_4 concentrations, we hypothesize that feeding a diet containing the NRC-recommended amount of I (CON), or feeding 113 g ASCO meal (HI) may have improved ruminal development via supplying the ideal amount of I for the fetus and improving efficiency of TH synthesis, or by ASCO meal providing bioactive compounds to the calf in-utero which increased rumen maturation.

Xylose challenge data including plasma xylose and glucose over 12 h on d 5 of life is presented in Table 8. Plasma xylose absorption on d 5 of life did not differ among treatments. Plasma glucose absorption, measured simultaneously with xylose, on d 5 of life decreased linearly ($P = 0.01$) with the addition of ASCO meal to the dam's diet (Table 8). During the xylose challenge on d 5 of life, plasma glucose absorption of calves decreased with incremental ASCO meal supplementation to prepartum diets, despite plasma xylose absorption being unaffected. D-xylose is used to measure intestinal absorptive capacity due to the intestinal receptors having less affinity towards D-xylose compared with D-glucose, therefore allowing for

less D-xylose absorption (Scharrer and Grenacher, 2000). Therefore, as ASCO meal was added to the diet of the dam, it appears that calf glucose receptors became more selective against glucose entry into the intestinal barrier. Calf BW gain had a tendency to be lower with addition of ASCO meal to the dam's diet, which could have been related to decreased glucose uptake from milk replacer, providing less nutrients for growth. Thyroid hormone metabolism is known to influence glucose turnover, as well as glucose metabolism, via insulin, especially during late gestation and when animals are neonates (Boelen, 2009; Hammon et al., 2012). It has been found that poor conversion of T_4 to T_3 around 9 d prior to birth resulted in decreased endogenous gluconeogenesis when calves were born (Steinhoff-Wagner et al., 2011), likely due to TH, particularly T_3, impacting glycogen storage (Boelen, 2009). However, in our study TH was relatively unaffected by treatments, therefore calf TH concentrations were possibly unrelated to decreased neonatal glucose absorption, and the glucose response could have been associated with in-utero effects of ASCO meal.

There may be benefits to feeding I as a seaweed source rather than an inorganic source to prepartum dams due to presence of bioactive compounds, however further research should be done to determine the ideal dosage of supplementation, the most effective seaweed species at stimulating an immune response, and whether seaweed should be fed as a meal or as a fermented product.

Conclusion

In summary, feeding the highest amount of ASCO meal as an I source rather than EDDI resulted in greater colostrum fat concentration, and higher calf initial BW, as well as tendencies

for greater skeletal growth and IgG AEA. The presence of phlorotannins and compounds that stimulate the immune system in ASCO meal may benefit colostrum composition and calf health over feeding inorganic I. Incremental supplementation of ASCO meal had little effect on calf plasma TH concentrations, and caused a decrease in neonatal glucose absorption, possibly due to increasing I concentration in the diet or via bioactive compounds. Based on the results of this study, feeding ASCO meal as an I source does not appear to have negative effects on calf metabolism and IgG absorption. It also did not negatively impact colostrum yield and composition, or cause high I passage into colostrum from the blood of the dam. It appears that ASCO meal can be used as a safe feed supplement for prepartum cows; however, more research is needed on how ASCO meal and other seaweeds interact with inorganic I in the diet. Additionally, further research is needed to understand the ideal seaweed species and amount to feed, how the compounds in ASCO meal interact with other compounds in the diet and the animal, and what specific components of seaweeds are driving biological responses in the animal. Producers should also carefully monitor the basal I concentrations in their diets, as adding seaweed to the diet typically increases I above requirements.

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Table 1. Nutrient analysis of milk replacer

Item	DM (%)
Crude fat	16.5 ± 0.1
CP	25.3 ± 0.35
Ash	6.81 ± 0.12

Table 2. Nutrient analysis of starter

Item	DM (%)
DM, % of fresh matter	0.88 ± 0.01
CP	28.2 ± 1.25
ADF	6.90 ± 0.75
NDF	15.1 ± 2.15
Fat	5.60 ± 0.36
Ash	7.28 ± 0.35
Starch	20.8 ± 2.99
NFC	43.8 ± 3.46
Ca	0.85 ± 0.05
P	0.68 ± 0.06
Mg	0.28 ± 0.02
K	1.30 ± 0.07
Na	0.16 ± 0.01
I (mg/kg)	4.44 ± 1.36

Table 3. Colostrum yield and composition from cows supplemented incremental amounts of *Ascophyllum nodosum* (ASCO) meal or EDDI.

	Treatment[1]				SEM	*P*-value[2]		
Item	CON	LO	HI	EDDI		Linear	Quadratic	HI vs. EDDI
Yield (kg)[3]	8.41	6.15	6.86	5.62	0.25	0.41	0.34	0.49
Fat (%)	4.42	4.54	6.63	4.14	0.87	0.07	0.33	0.04
Fat yield (g)[3]	447	280	415	233	2.78	0.84	0.22	0.19
Total protein (%)	15.7	15.7	14.7	16.3	0.94	0.47	0.65	0.22
Total protein yield (g)	1,392	1,004	1,074	916	180	0.21	0.28	0.53
True protein (%)	15.2	15.2	15.2	15.9	0.98	0.97	0.96	0.61
True protein yield (g)	1,351	969	1,043	893	175	0.21	0.27	0.54
Total Solids (%)	25.4	24.2	26.5	24.5	1.35	0.57	0.32	0.31
Total solids yield (g)	2,261	1,634	1,869	1,434	318	0.38	0.25	0.33
Iodine (ng/mL)	179	241	235	342	46.8	0.37	0.53	0.11
Iodine yield (mg)	1.88	1.60	2.19	2.04	0.49	0.66	0.48	0.83
IgG (g/L)[3]	103	98	98	110	0.59	0.77	0.85	0.50
IgG yield (g)	757	639	790	595	111	0.83	0.31	0.20

[1]CON = 0.5 mg I/kg intake, LO = CON + 56.7 g ASCO meal, HI = CON + 113.4 g ASCO meal, EDDI = CON + 124.8 mg I

[2]Single df contrasts were used to test (1) linear and quadratic effects in response to incremental amounts of ASCO meal supplementation, and (2) HI vs. EDDI treatments

[3]Data were transformed to their square root, statistically analyzed, and then transformed back. SEM presented were not transformed back.

Table 4. Number of calves per experimental treatment treated for illness.

	Experimental Treatment			
Medical treatment	CON	LO	HI	EDDI
Electrolytes	2	2	0	4
Probiotic Paste	1	1	0	1
Vitamin injection	0	0	0	1
Bloat release	0	0	0	1

Table 5. Intakes, body weights (BW), skeletal measurements, average daily gain (ADG), and feed efficiency of calves born to dams supplemented incremental amounts of *Ascophyllum nodosum* (ASCO) meal or EDDI.

	Treatment[1]				SEM	*P*-value[2]				
Item	CON	LO	HI	EDDI		Linear	Quadratic	HI vs. EDDI	Trt x Wk	Wk
Starter intake (kg/d)	0.55	0.51	0.47	0.48	0.05	0.27	0.97	0.94	0.67	<0.001
Milk replacer intake (kg/d)	0.59	0.60	0.60	0.59	0.01	0.61	0.20	0.52	0.55	<0.001
DMI (kg/d)	1.15	1.11	1.05	1.07	0.05	0.19	0.93	0.81	0.48	<0.001
BW										
Weekly BW (kg)	60.2	58.0	58.8	56.4	1.53	0.54	0.43	0.26	0.11	<0.001
Initial BW (kg)	45.0	43.4	45.4	41.8	1.10	0.77	0.18	0.02	-	-
Final BW (kg)	81.2	76.9	77.2	76.3	2.09	0.19	0.36	0.75	-	-
BW gain (kg)	36.3	33.5	31.8	34.5	1.83	0.10	0.81	0.29	-	-
Hip height										
Weekly hip height (cm)	89.0	88.1	88.9	87.2	0.67	0.88	0.29	0.09	0.86	<0.001
Initial hip height (cm)	83.9	82.0	83.2	82.1	0.90	0.58	0.17	0.40	-	-
Final hip height (cm)	94.4	93.3	94.6	92.5	0.66	0.86	0.15	0.03	-	-
Hip height gain (cm)	10.5	11.3	11.5	10.4	0.96	0.33	0.68	0.27	-	-
Withers height										
Weekly withers height (cm)	84.3	84.1	84.7	83.0	0.62	0.67	0.59	0.05	0.14	<0.001
Initial withers height (cm)	79.5	78.9	79.6	78.1	0.75	0.99	0.49	0.18	-	-
Final withers height (cm)	90.3	89.5	90.1	88.2	0.70	0.86	0.40	0.06	-	-
Withers height gain (cm)	10.8	10.6	10.6	10.1	0.63	0.83	0.90	0.61	-	-
Heart girth										
Weekly heart girth (cm)	95.8	94.7	94.6	93.0	0.81	0.32	0.64	0.17	0.92	<0.001
Initial heart girth (cm)	86.7	86.6	86.0	84.6	0.90	0.61	0.84	0.26	-	-
Final heart girth (cm)	105	104	104	102	0.91	0.27	0.43	0.17	-	-
Heart girth gain (cm)	18.7	17.2	17.7	17.5	0.87	0.41	0.35	0.88	-	-
Body Length										
Weekly body length (cm)	70.9	70.7	71.7	70.4	0.82	0.54	0.53	0.28	0.58	<0.001
Initial body length (cm)	62.4	62.5	64.3	63.1	1.01	0.18	0.49	0.37	-	-
Final body length (cm)[3]	79.3	78.6	79.7	78.1	11.1	0.71	0.35	0.15	-	-

Body length gain (cm)	16.8	16.1	15.5	15.0	0.91	0.32	0.96	0.68	-	-
Hip width										
Weekly hip width (cm)	22.0	22.0	21.7	21.8	0.34	0.65	0.72	0.91	0.99	<0.001
Initial hip width (cm)	19.7	19.8	19.5	19.9	0.45	0.78	0.81	0.56	-	-
Final hip width (cm)	24.4	24.2	24.2	23.9	0.34	0.65	0.76	0.53	-	-
Hip width gain (cm)	4.67	4.44	4.70	4.50	0.39	0.96	0.62	0.72	-	-
ADG (kg/d)	0.70	0.68	0.65	0.71	0.04	0.37	0.92	0.27	0.15	<0.001
Feed efficiency (ADG/DMI)	0.74	0.77	0.84	0.93	0.13	0.60	0.89	0.63	0.98	<0.001

[1]CON = 0.5 mg I/kg intake, LO = CON + 56.7 g ASCO meal, HI = CON + 113.4 g ASCO meal, EDDI = CON + 124.8 mg I

[2]Single df contrasts were used to test (1) linear and quadratic effects in response to incremental amounts of ASCO meal supplementation, and (2) HI vs. EDDI treatments

[3]Data were squared, statistically analyzed, and then transformed back.

Table 6. Plasma thyroid hormones and serum IgG of calves born to dams supplemented incremental amounts of *Ascophyllum nodosum* (ASCO) meal or EDDI.

	Treatment[1]				SEM	*P*-value[2]				
Item	CON	LO	HI	EDDI		Linear	Quadratic	HI vs. EDDI	Trt x D	D
Thyroid Hormone										
Weekly total T_4(µg/dL)	12.80	11.67	12.67	12.68	0.36	0.81	0.02	0.99	0.78	<0.001
Total T_4 0 h (µg/dL)	18.48	16.6	18.17	19.27	1.18	0.85	0.24	0.50	-	-
Total T_4 24 h (µg/dL)	19.58	19.42	20.37	18.17	0.96	0.57	0.64	0.11	-	-
Weekly total T_3 (ng/mL)	2.75	2.55	2.55	2.76	0.14	0.33	0.54	0.30	0.52	<0.001
Total T_3 0 h (ng/mL)	2.79	3.40	3.08	3.07	0.50	0.68	0.45	0.98	-	-
Total T_3 24 h (ng/mL)	6.13	5.05	5.46	5.92	0.44	0.29	0.17	0.44	-	-
Weekly free T_4 (ng/dL)	1.24	1.20	1.25	1.21	0.04	0.86	0.28	0.48	0.99	<0.001
Free T_4 0 h (ng/dL)	1.30	1.21	1.38	1.23	0.10	0.58	0.29	0.30	-	-
Free T_4 24 h (ng/dL)	1.84	1.91	1.97	1.85	0.11	0.39	0.97	0.44	-	-
Weekly free T_3 (pg/mL)	4.44	4.39	4.37	4.30	0.20	0.81	0.95	0.80	0.95	<0.001
Free T_3 0 h (pg/mL)	4.12	4.25	4.61	3.97	0.46	0.45	0.84	0.33	-	-
Free T_3 24 h (pg/mL)	7.59	7.28	7.45	7.38	0.42	0.82	0.65	0.91	-	-
Weekly total T_4/T_3 ratio (ng/dL)	56.8	52.8	57.8	55.4	1.97	0.73	0.06	0.39	0.59	<0.001
Total T_4/T_3 ratio 0 h (ng/dL)	72.8	55.9	59.7	67.0	6.62	0.16	0.19	0.42	-	-
Total T_4/T_3 ratio 24 h (ng/dL)	31.4	35.8	36.1	31.7	2.71	0.23	0.54	0.25	-	-
Weekly free T_4/T_3 ratio (ng/dL)	2.94	2.83	2.90	2.91	0.10	0.73	0.42	0.89	0.97	<0.001
Free T_4/T_3 ratio 0h (ng/dL)	3.27	2.95	3.21	3.21	0.19	0.84	0.22	0.98	-	-
Free T_4/T_3 ratio 24 h (ng/dL)	2.49	2.50	2.62	2.36	0.14	0.50	0.72	0.17	-	-
IgG									-	-
0 h IgG (g/L)	0.42	0.82	0.47	0.42	0.18	0.85	0.11	0.84	-	-
24 h IgG (g/L)	24.8	27.2	26.2	24.9	1.16	0.41	0.25	0.43	-	-
AEA (%)[3]	33.6	35.5	35.4	30.8	1.69	0.46	0.66	0.06	-	-

[1]CON = 0.5 mg I/kg intake, LO = CON + 56.7 g ASCO meal, HI = CON + 113.4 g ASCO meal, EDDI = CON + 124.8 mg I

[2]Single df contrasts were used to test (1) linear and quadratic effects in response to incremental amounts of ASCO meal supplementation, and (2) HI vs. EDDI treatments

[3]AEA = [(serum IgG at 24-h (g/L) x BW (kg) x 0.09) / colostral IgG intake (g)] x 100 (Quigley et al., 1998)

Table 7. Plasma beta hydroxybutyrate (BHB) of calves born to dams supplemented incremental amounts of *Ascophyllum nodosum* (ASCO) meal or EDDI.

	Treatment[1]				SEM	*P*-value[2]				
Item	CON	LO	HI	EDDI		Linear	Quadratic	HI vs. EDDI	Trt x Wk	Wk
Weekly BHB (mmol/L)	0.24	0.21	0.23	0.21	0.01	0.46	0.02	0.21	0.25	<0.001
Initial BHB (mmol/L)	0.17	0.18	0.18	0.20	0.02	0.75	0.97	0.52	-	-
Final BHB (mmol/L)	0.33	0.27	0.29	0.27	0.02	0.12	0.05	0.41	-	-

[1]CON = 0.5 mg I/kg intake, LO = CON + 56.7 g ASCO meal, HI = CON + 113.4 g ASCO meal, EDDI = CON + 124.8 mg I

[2]Single df contrasts were used to test (1) linear and quadratic effects in response to incremental amounts of ASCO meal supplementation, and (2) HI vs. EDDI treatments

Table 8. Xylose challenge data from calves born to dams supplemented incremental amounts of *Ascophyllum nodosum* (ASCO) meal or EDDI. Plasma xylose and glucose were measured on d 5 of life over a 12 h period.[1]

	Treatment[2]				SEM	*P*-value[3]				
Item	CON	LO	HI	EDDI		Linear	Quadratic	HI vs. EDDI	Trt x H	H
Xylose (mmol/L)	2.03	2.13	2.08	2.05	0.07	0.56	0.36	0.72	0.98	<0.001
Glucose (mg/dL)	105.00	101.07	93.67	97.54	2.82	0.01	0.61	0.33	0.39	<0.001

[1]Calves were fed 0.5 g D-xylose/ kg body weight and fasted for 12 h to measure intestinal absorption (Merritt and Duelly, 1983)

[2]CON = 0.5 mg I/kg intake, LO = CON + 56.7 g ASCO meal, HI = CON + 113.4 g ASCO meal, EDDI = CON + 124.8 mg I

[3]Single df contrasts were used to test (1) linear and quadratic effects in response to incremental amounts of ASCO meal supplementation, and (2) HI vs. EDDI treatments

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IACUC #: 210203
Project: Discovering Potential Methane Mitigation, Animal Health Benefits, Economic Hurdles, and Outreach Opportunities to Improve Adoption of Algae-Feed

Approval Date: 09-Jul-2021

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category C in Section V of the Application for Review of Vertebrate Animal Use in Research or Instruction - Animal use activities that involve either no pain or potentially involve momentary, slight pain, discomfort or stress not requiring the use of pain relieving drugs or methods.

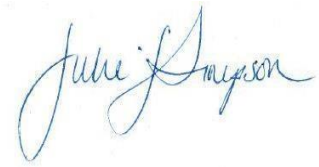
Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

Please Note:

1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. Information about the program, including forms, is available at http://unh.edu/research/occupational-health-program-animal-handlers.

If you have any questions, please contact either Dean Elder at 862-4629 or Susan Jalbert at 862-3536.

For the IACUC,



Julie Simpson, Ph.D.
Director
cc: File