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KELP MEAL (Ascophyllum nodosum) SUPPLEMENTATION TO ORGANIC LACTATING DAIRY COWS: EFFECTS ON MILK PRODUCTION, MILK COMPOSITION, ANIMAL HEALTH AND NUTRIENT UTILIZATION DURING THE NON-GRAZING AND GRAZING SEASONS IN NEW HAMPSHIRE

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TABLE OF CONTENTS

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LIST OF TABLES AND FIGURES

ABSTRACT

KELP MEAL (*Ascophyllum nodosum)* SUPPLEMENTATION TO ORGANIC LACTATING DAIRY COWS: EFFECTS ON MILK PRODUCTION, MILK COMPOSITION, ANIMAL HEALTH AND NUTRIENT UTILIZATION DURING THE NON-GRAZING AND GRAZING SEASONS IN NEW HAMPSHIRE

by

Nicole Antaya

University of New Hampshire, September, 2016

This thesis examines the feeding of kelp meal (KM), most commonly produced from the species *Ascophyllum nodosum* to lactating dairy cattle. In the first experiment KM was fed at incremental levels to lactating jersey cattle over the winter season in Lee, New Hampshire. The objective of this study was to investigate the effects of incremental amounts (0, 57, 113, and 170 g/d) of KM on animal performance and milk iodine concentration in dairy cows fed high-forage diets. While animal performance was not improved, milk iodine output increased linearly in cows fed KM. Trends and quadratic effects were observed for nutrient digestibility, and plasma concentration of nonesterified fatty acids reduced linearly with KM supplementation.

In the second experiment treatments of 0 g and 113 g KM were fed to lactating Jersey cattle over the grazing season in Lee, New Hampshire. The objective of this second experiment was to determine how feeding 113 g of KM impacts animal performance and milk iodine concentration lactating dairy cattle during the grazing season in the Northeast. The results of this study found animal performance was not improved with KM supplementation. An increase in

milk iodine levels were observed in cows fed KM. Somatic cell count was observed to be lower with KM feeding but was not statistically analyzed.

CHAPTER I

LITERATURE REVIEW

Kelp meal (**KM**) is a popular feed supplement used on organic dairy farms in the Northeast. This thesis examines the impact of kelp meal on lactating organic dairy cows during the winter and summer/grazing season. The objective of this thesis is to provide a comprehensive understanding of the potential impacts of this supplement on animal performance, health, and metabolism as well as its potential effects on milk quality, including milk yield, components and milk iodine concentration.

Due to the seasons experienced in the Northeast pasture is only available for approximately 6 months of the year or less. During the winter months the major forage source in the diet comes from stored forages (e.g., baleage, hay, haylage, silage) which are made and preserved during the summer season. Differences in plant species and nutrient composition between pasture and stored forages can impact how supplements are used by the dairy cow and how they may affect milk production and composition. Therefore, it is important to examine the impacts of supplements both during the winter months when cows are consuming preserved forages and during the grazing season when cows are consuming pasture. In addition, animals on pasture are at increased risk of higher levels of stress due to heat, increased exercise, parasites, and flies. Thus, it is important to understand if KM is effective at reducing the stress response in grazing dairy cows.

Kelp Meal Description and Use

Kelp meal is produced from dried and ground seaweed. While numerous seaweed species including *Ascophyllum nodosum, Laminaria digitata*, and *Macrocystis pyrifera* may be used to produce KM, *Ascophyllum nodosum* is the most commonly used in agriculture and was the species present in the KM used in this thesis. *Ascophyllum nodosum*, popularly known as "rockweed" or "Norwegian kelp", is a brown algae species found in the intertidal zone on the shores of the North Atlantic. *Ascophyllum nodosum* is a slow growing, long lived species (Seeley et al., 2012). Rockweed growth is impacted by water velocity and grows longer in places where water is calm (Rockweek Fishery Management Plan, 2013). Rockweed age, which is estimated by length of longest shoot, can be difficult to determine accurately as shoots are continuously broken by ocean currents (Rockweed Fishery Management Plan, 2013). Some studies have estimated maximum lifespans of 60 years (Rockweed Fishery Management Plan, 2013), while other evidences suggest that the holdfasts for these plants may live up to 400 years. According to Seeley et al. (2012) the harvesting of *A. nodosum* is among the top methods of human impact on coastal habitats. *A. nodosum* forms marine forests similar to terrestrial counterparts and provides habitat for over 150 species including species of birds, fish, and marine invertebrates some of them are endangered or are at risk of becoming so (Seeley et al., 2012). Some fish and invertebrate species which rely on *A. nodosum* as a habitat are also of economic importance particularly in Maine where a large portion of the population relies on the fishing and shell fishing industries as a source of income. The sustainability of *A. nodosum* harvesting remains a concern and an area in need of more research. Many scientists in the marine community believe that *A. nodosum* and the ecosystem it creates are under threat by commercial harvesting and that more regulations need to exist to protect this species, specifically in Maine where harvesting

regulations are minimal (Seeley et al., 2012). In addition to the potential effect for KM to enhance dairy cow health and performance, the sustainability of harvesting *A. nodosum* must be further examined.

Kellog et al. (2006) examined the effects of feeding KM or no KM in a TMR to a herd of approximately 600 lactating dairy cattle in an on-farm study lasting 3 months. Cows of multiple breeds and parity were examined in this study. Large breed cows included Holsteins, Ayrshires, and Brown Swiss while small breed cows included Jerseys, Milking Shorthorns, Holstein/Jerseys crosses and some smaller first lactation animals of the larger breeds. Cows on the KM treatment were supplemented KM at a rate of 0.25% of diet DM which was approximately 111.4 g/d for large cows and approximately 95.7 g/d for small cows. Milk production and components were recorded. In addition, 15 cows were randomly selected weekly for heat stress measurements. Milk yield and reproductive performance were improved in large cows supplemented with KM but not in the smaller animals. Cows of both sizes fed KM had lower respiration rate (breath/min) than control cows on 4 of the 8 weekly collection times throughout the study. Cvetkovic et al. (2004) examined feeding KM or a control diet to 24 lactating Holstein cows. Kelp meal was supplemented at a rate of 113.4 g/d for 1 week and 56.7 g/d for the remainder of the study. Milk yield and milk protein yield were improved with KM supplementation. However, DMI was not affected with KM supplementation indicating that KM improved feed efficiency. On the other hand, these authors reported no effect of KM supplementation on reducing respiration rate, rectal temperature, or rear udder temperature indicating KM was not effective at mitigating heat stress.

Berry and Turk (1944) examined the impacts of feeding a supplement made from dried and ground *Marcocystis pyrifera*, commonly known as “giant kelp” on growth rate, breeding

efficiency, milk production, and intake of dairy heifers and primiparous cows. Seventy eight heifers were used in the first year of the study and 50 of these animals remained on the study for the second year. In the first year of the study, heifers from 12 months of age to first calving were assigned to treatments containing 0 or 464.6 g of giant kelp meal. No differences between treatments were found in growth rate or breeding efficiency. Calves born from dams fed giant kelp meal were slightly heavier than calves born from dams on the control treatment. In the second year of the study, 50 of the original 78 animals remained on their treatments through the first lactation and until their second calving. No differences were found for milk production, DMI, and breeding efficiency. Calves born from dams fed giant kelp meal were slightly lighter than calves born to dams on the control treatment, the opposite of what was observed in the first year calving. Potential limitations in this study were the use of multiple breeds (Holsteins, Aryshires, Gurenseys, and Jerseys) and the lack of modern statistical analysis. Dairy management and feeding systems have changed significantly since this study was conducted in the mid 40's, therefore, there is a demand for more research in the area of KM supplementation. Additionally, Berry and Turk (1944) examined the feeding of giant kelp meal while the vast majority of currently commercially available kelp meals are produced from *A. nodosum.*

A study by Karatzia et al. (2012) examined the effect of KM in the form of dried and ground *A. nodosum* on hematological parameters of 19 lactating Holstein dairy cattle. Cows were blocked by milk production and assigned to treatments of 0 or 80 g of KM. Weekly blood samples were collected from the cattle and analyzed for hemoglobin, packed cell volume, erythrocyte and leukocyte count, and plasma concentrations of glucose and sorbitol dehydrogenase. Cows were allowed 3 weeks of adjustment to treatments prior to the start of weekly sampling. This study found no significant effect of KM on milk yield or milk

components. The only hematological parameters reported to be significant were the concentrations of glucose and sorbitol dehydrogenase. Blood glucose was significantly higher for cows fed KM in weeks 2, 4, 5, 6, and 7, (P-value < 0.05) whereas sorbitol dehydrogenase was significantly lower for cows fed KM in weeks 3, 4, 5, 6, and 7 (P-value < 0.01). The effect of KM on both glucose and sorbitol dehydrogenase concentrations became gradually more pronounced over the duration of the study. Some hypotheses were suggested to explain why KM had an effect on plasma glucose concentration: 1) KM may increase propionate production in the rumen, and 2) KM may increase gut microflora resulting in improved digestion and better nutrient utilization, thus allowing increased glucose absorption by the animal. Sorbitol dehydrogenase is an enzyme involved in carbohydrate metabolism and should be low in the blood of healthy animals while elevated blood sorbitol dehydrogenase values may indicate hepatocellular injury (Karatzia et al 2012). Therefore, results from Karatzia et al. (2012) suggest that KM may have a protective effect on the liver cells of the dairy cattle. Results from Karatzia et al. (2012) also indicate the need for examination of long-term kelp meal supplementation because the effects of KM on the plasma concentrations of glucose and sorbitol dehydrogenase became more pronounced over time.

Erickson et al. (2012) examined the short-term preference of dairy calves to KM, in the form of dried and ground *A. nodosum*, based on the hypothesis that the high glutamic acid content in KM could stimulate intake as consumption of monosodium glutamate stimulates intake in humans. Post weaning Holstein heifers were individually housed with access to a manger, which allowed heifers the choice of 3 treatments, randomly assigned at each feeding: 0 g (control), 30 or 60 g of KM. Treatment preference was determined by measuring the calves' intake of each of the 3 offered treatments, the treatment with the greatest intake was associated

with greatest preference. Calves remained on trial for 7 d, the first 2 d were used for diet adaptation, and during d 3 to 5 heifers were allowed access to all 3 treatments to determine the animals first choice treatment preference. The last 2 d of the trial (d 6 and 7) the first choice treatment preference was removed in order to determine the animals second choice treatment preference. Heifers consistently selected the control treatment (i.e., 0 g of KM) as their first preference (*P*-value = 0.004) with no significant difference (*P*-value = 0.22) in preference between 30 or 60 g of KM supplementation. These results suggested that the significantly reduced preference for KM by dairy heifers may negatively impact feed intake potentially leading to decreased growth.

Fike et al. (2004) examined impacts of *A. nodosum* extracts supplementation on ruminal parameters in lambs under heat stress and thermo-neutral conditions. Three treatments were used: 1) a control diet, 2) a diet containing hay treated with A. nodosum extract prior to harvest, and 3) a diet including 1% (DM basis) A. nodosum extract over a period of heat stress and a period of thermo-neutral conditions. Results of this study found that direct supplementation A. nodosum extract (diet 3) improved both N and OM digestibility ($P < 0.05$) when compared to control and diet 2. This study also examined ruminal pH and VFA concentration and observed that butyrate concentrations were reduced with both forms of supplementation (10.3, 9.2 and 8.8 m*M* for control, treated hay, and direct supplementation, respectively). On the other hand, total VFA concentration as well as concentrations of acetate, propionate, isobutyrate, and isovalerate were not significantly impacted by treatments. The results of Fike et al. (2004) study provide additional evidence that *A. nodosum*, or extracts of *A. nodosum* had some impact on ruminal fermentation.

Iodine

Kelp meal is marketed to livestock producers as a mineral supplement rich in trace minerals. Kelp meal analyzed in the present thesis had an average ash concentration of approximately 26% with iodine found as one the minerals with the highest concentrations (Table 2). In the environment, iodine is present in the forms iodide and iodate. These anions dissolve readily in water and, therefore, are not very stable in the soil. Iodine in these forms is leached from the soil via rainwater and runs off into the ocean, resulting in seawater with high concentrations of iodine. In seawater, iodine may be oxidized and returned to the atmosphere, a process which occurs slowly or taken up by marine plants (Bowman and Russell, 2006). Due to slow iodine cycling from oxidation, marine plants have a highly available supply of iodine in the surrounding seawater which they readily uptake iodine causing these plants to have higher levels of this element than their terrestrial counterparts. Levels of iodine in terrestrial plants are highly variable and depend on species, proximity to the ocean, stress level placed on the plant, climate, and weather patterns (Borucki Castro et al., 2011). Terrestrial plants raised in soils of high iodine concentrations are also capable of up taking iodine in similar concentrations to marine species (Bowman and Russell, 2006).

In feed for animals as well as food for humans iodine is also present as iodide and iodate (Bowman and Russell, 2006). In the human digestive tract iodide is the form which may be absorbed by the small intestine while iodate must be reduced to iodide in order to be absorbed (Bowman and Russell, 2006). Iodine in the bloodstream may be absorbed by the thyroid gland for the production of the thyroid hormones or taken up by the kidney and excreted via urine. Concentrations of the thyroid hormones impact how iodine is metabolized. For instance, if thyroid hormone levels are adequate excess iodine is excreted via urine, but if thyroid hormone

levels need to be increased for meeting specific metabolic functions more iodine is taken up by the thyroid gland to produce these hormones (Saladin, 2010).

In the body, iodine taken up by the thyroid gland is used in the production of the thyroid hormones triiodothyronine (**T_3**) and thyroxine (**T_4**). The thyroid hormones control metabolism and are essential in maintaining homeostasis. High levels of thyroid hormones result in increased heart and respiratory rates, body temperature, appetite, and nutrient metabolism while low levels of thyroid hormones result in a decrease of these bodily functions (Saladin, 2010). In addition, thyroid hormones promote growth by stimulating the pituitary gland to release growth hormone, which has a widespread effect on almost all cells in the body. In the developing fetus thyroid hormones are essential for growth as well as the development of the nervous system (Saladin, 2010).

Production of the thyroid hormones as well as growth of the thyroid gland is regulated by the thyroid stimulating hormone (**TSH**) produced in the pituitary gland. Thyroid stimulating hormone is regulated by the hormone thyrotropin-releasing hormone (**TRH**), which is secreted by the hypothalamus. Production of the thyroid hormones is controlled by a negative feedback loop where low levels of thyroid hormones trigger TSH production, which stimulates thyroid hormone synthesis while high levels of thyroid hormones decrease TRH and TSH synthesis reducing the production of the thyroid hormones (Saladin, 2010). Formation of the thyroid hormones requires thyroglobulin, the carrier protein of iodine in the thyroid gland, and the enzyme thyroid peroxidase that modifies thyroglobulin to form monoiodotyrosine and diiodotyrosine, which are the precursors of the thyroid hormones. Two diiodotyrosine molecules are linked together to form the hormone T_4 while 1 diiodotyrosine and 1 monoiodotyrosine molecule are linked together to form the hormone T_3 (Bowman and Russell, 2006).

Mammals concentrate iodine into milk in order to provide iodine to their offspring as iodine is essential for growth and development (Bowman and Russell, 2006). Therefore, dairy products are an important source of iodine for the human population. Bread made with iodate conditioners used to prolong shelf life may be another source of iodine for the human population. However, growing concerns about high bread iodine content has caused many commercial bread producers to switch to non-iodine conditioners, thus removing this source of iodine from the population (Pearce, 2004). Iodized salt is another source of iodine for the human population and remains one of the most important mechanisms of preventing iodine deficiency. However, because of increasing health concerns including high blood pressure and obesity many doctors and nutritionists have been promoting low sodium diets, potentially reducing iodine intake by limiting salt intake (Pearce, 2004).

Goiter or swelling of the thyroid gland can result from a variety of thyroid conditions including Graves disease, in which the thyroid is hyperstimulated due to the body mimicking effects of TSH. However thyroid disorders aside, goiter remains the most recognized physical indicator of iodine deficiency, and goiter due to iodine deficiency is known as endemic goiter (Saladin, 2010). Goiter occurs when the sensitive balance between TSH and the production of thyroid hormones is disrupted. In the case of iodine deficiency low thyroid hormone levels stimulate an increase in TSH, but with no available iodine to produce the thyroid hormones, TSH release continues because it is not negatively inhibited by the presence of thyroid hormones. This continued TSH production results in swelling of the thyroid gland leading to goiter (Saladin, 2010).

Iodine deficiency is the leading preventable cause of mental retardation worldwide (Bowman and Russell, 2006). Iodine is essential in development from the second trimester to the

third year of life where it is necessary for development of nerves and myelination of the central nervous system (Bowman and Russell, 2006). Even mild iodine deficiency can impair cognitive development, which may result in decreased school and work performance in children and may decrease overall social and economic status of the individual as well as the country as a whole (Andersson et al., 2005). Therefore, close monitoring of iodine consumption by humans particularly pregnant women and young children is essential (Andersson et al., 2005).

Urinary iodine is used to measure iodine status. The World Health Organization compiled urinary iodine status surveys from a variety of sources including scientific literature, country offices, U.N. affiliates, and health ministry's to determine the worldwide iodine status of school children between 1993 and 2003 and between 1997 and 2006. (Benoist et al. 2008) The most recent data (1997 to 2006) on urinary iodine concentration of school age children worldwide was published by Benoist et al. (2008) and represented 130 countries and 762.6 million school age children amounting to approximately 91.1% of the world's school age children. Urinary iodine data indicated that 31.1% or 266 million school children worldwide had insufficient iodine intake. The highest number of children with less than adequate iodine intake were found in Southeast Asia (73.1 million) followed by Africa (57.7 million). Europe had the highest proportion of children with insufficient iodine intake (52.4%). The lowest incidence of iodine deficiency was the Americas with 12 million children potentially suffering from iodine deficiency. While these results indicate that approximately a third of school children worldwide consume insufficient iodine, this number has decreased by about 5% since the 2003 survey (Andersson et al., 2005; Benoist et al., 2008).

Although most humans appear tolerant to high levels of dietary iodine, there can be adverse health effects of consuming iodine in excess, particularly in individuals with underlying

thyroid conditions (Pennington, 1990). The upper limit of iodine intake likely to cause no negative health effects is 1,100 µg/d for adults and 200 µg/d in children 1 to 3 years of age. The subclinical effects of iodine excess may include short term decreased levels of thyroid hormones (Zimmerman et al., 2005). Clinical negative impacts on health include goiter, hypothyroidism, and hyperthyroidism (Pennington, 1990). Hyperthyroidism which may result from iodine excess causes increased metabolism leading to weight loss, tachycardia, and muscle weakness (Bowman and Russell, 2006). Zimmerman et al. (2005) measured thyroid volume in 3,000 children ages 6-12, to determine if consumption of iodine in excess of the recommended allowance led to an increase in thyroid volume. The results of that survey found iodine intakes exceeding 500 µg/d in one population of children (n=280) living in coastal Japan, these children were found to have an increase in thyroid volume compared to the other children surveyed. They found no increase in thyroid volume in children consuming 300 to 500 µg/d although this amount is still over the recommended daily allowance for children (Zimmerman et al., 2005).

A positive correlation between dietary iodine intake by dairy cows and milk iodine has been established. For instance, Berg et al. (1988) fed varying amounts (0 to 45 mg/head/d) of ethylenediamine dihydroiodide (**EDDI**), a commonly used iodine supplement in dairy cattle rations, and reported a positive correlation ($r = 0.92$) between EDDI intake and bulk tank milk iodine indicating that iodine from EDDI is readily absorbed and secreted in milk. In a survey of 60 Canadian dairy farms, 86% of the farms were feeding iodine in excess of the requirements of dairy cattle (Borucki Castro et al., 2011). In the Burucki Castro et al. (2011) survey, samples of feed components, bulk tank milk, and water were analyzed for iodine content. Forage sources accounted for approximately 17% of the iodine necessary for meeting the requirements of dairy cows while concentrates contained small concentrations of iodine and were not considered a

source significant towards meeting the cows' iodine requirements (Borucki Castro et al., 2011). Therefore, approximately 83% of the iodine necessary for dairy cow maintenance was supplied by mineral mixes. According to these data, mineral mixes are the major source of iodine for dairy cattle and over supplementing minerals may lead to high levels of iodine in milk.

Borucki Castro et al. (2012) conducted 2 experiments to examine both the impacts of iodine supplementation as well as post-milking teat dipping practices on milk iodine concentrations. In the first experiment, 63 lactating Holsteins were assigned to treatments in a 3 × 3 factorial design. Dietary treatments were 0.25, 0.5, and 1 mg/kg DM iodine in the form of EDDI. Post-milking teat dipping treatments were chlorhexidine dip (0% iodine), 1% iodine dip, and 1% iodine spray. Milk production as well as milk fat, protein, and iodine concentrations were analyzed. Blood samples were taken to be analyzed for T_4. This study found a positive linear effect between level of dietary iodine supplementation and milk iodine concentration. Milk iodine reached levels of 301, 414, and 482 µg/kg for the 0.25, 0.5, and 1 mg/kg EDDI, respectively. No significant difference was found between the 0% iodine post-dip (246 µg/kg) and the 1% iodine post-dip (495 µg/kg), however milk iodine was significantly higher when cows were sprayed with 1% iodine post-milking (655 µg/kg). This difference is believed to be attributed to the ability for the skin to absorb iodine. Spraying the teats rather than dipping requires more spray and covers a larger area, thus increasing the iodine present and the surface area over which it may be absorbed. There was no effect of dietary iodine treatments or teat dipping practice on T_4 concentrations. With such a strong correlation between iodine intake and milk iodine concentrations, cautions must be taken when formulating rations for dairy cattle to ensure milk quality and prevent excess iodine in milk. Currently, there is no maximum allowable

level of iodine in milk; however, it would be beneficial to regulate levels of milk iodine in order to prevent the population from being exposed to excess iodine intake.

Several studies (Pennington et al., 1990; Dahl et al., 2003; Pearce et al., 2004) have shown the variation in milk iodine concentrations between summer and winter months. Two hypotheses exist to explain this variation including: 1) levels of concentrate supplementation and 2) goitrogen presence in pasture species. It was hypothesized that the difference in milk I between summer and winter months could be attributed to differences in feeding between these seasons. When cows transition from winter to pasture feeding during the summer months, the rate of concentrate supplementation generally decreases, concentrates often contain vitamin and mineral mixes leading to the hypothesis that, when the level of dietary concentrates is reduced the level of vitamin and mineral mix is also decreased, possibly accounting for the reduction in milk iodine between summer and winter months (Dahl et al., 2003).

The second hypothesis is that the reduction in milk iodine in the summer months could be due to the presence of goitrogens found in pasture. Goitrogens are compounds which interfere with the absorption of iodine by the thyroid gland and may impair thyroid hormone production (Bowman and Russell, 2006). Several plant species relevant to dairy cattle nutrition contain goitrogens with potential to impair iodine metabolism or thyroid hormone production. White clover (*Trifolium repens*) is a cool season perennial legume commonly found in the Northeast U.S. Certain strains of white clover contain hydrocyanic acid, which is converted to thiocyanate, a known goitrogen, in the animal's body (Frame et al., 1998). Yong-An et al. (1984) fed ewes several forage species known to contain cyanogenic glycosides including legume species common in the Northeast; white clover and red clover (*Trifolium pretense*). While cyanogenic glycosides were found in all species, white clover had the highest concentration. Plasma and

urinary thiocyanate concentrations increased with increasing levels of cyanogenic glycosides in the plant and with duration of feeding (Yong-An et al., 1984).

Antimicrobial Activity

Ascophyllum nodosum and other brown algae species produce phlorotanins, which are polyphenolic compounds similar to terrestrial tannins (Li, 2013). Within brown algae species the biologically active phlorotannins are believed to protect the plant from stress, pathogens, radiation, and herbivory (Li, 2013). Based on their bioactive roles in the plant, phlorotannins are believed to act as antimicrobial and antioxidant compounds when ingested by animals.

The potential of *A. nodosum* to act as an antimicrobial was tested by Bach and Wang (2007). Thirty-two steers were inoculated with *Escherichia coli* O157:H7. At d 7 post inoculation steers were assigned to 1 of 4 treatments of dried and ground *A. nodosum*: 1) 0 g (control), 2) 10 g for 14 d, 3) 20 g for 7 d, or 4) 20 g for 14 d. Overall, concentrations of *E. coli* were significantly lower in fecal samples from cattle supplemented with *A. nodosum* at a rate of 10 g for 14 d and 20 g for 7 d when compared to the control treatment. There was no significant difference between the control and the supplementation rate of 20 g of *A. nodosum* for 14 d; this result was unexpected and may be attributed to individual animal variation in shedding of *E. coli*. Samples collected from fecal pats in pens that held *A. nodosum* supplemented steers were found to have less *E. coli* than fecal pat samples from the control steers pen. This reduction of *E.coli* in fecal pats of supplemented steers suggests that the antimicrobial action of *A. nodosum* on *E. coli* continues in the environment and, therefore, may help to reduce the spread of *E. coli* among animals. While this study demonstrates that dried and ground *A. nodosum* reduced fecal shedding of *E.coli*, it did not identified the specific compound responsible for this response, although it

was suggested that *A. nodosum* acts by altering conditions in the gastrointestinal tract making it less favorable for *E. coli.*

Wang et al. (2009) conducted an *in-vitro* experiment using isolated phlorotannins from *A. nodosum.* Isolated phlorotannins were cultured with 4 strains of *E. coli* at rates of 0, 25, 50 and 100 µg/mL. Phlorotannins prevented growth of all 4 strains of *E.coli* tested at rates of 50 and 100 µg/mL, and at 100 µg/mL the phlorotannins were considered bactericidal against all 4 strains. The action of phlorotannins was also compared to the action of terrestrial tannins including condensed tannins and hydrolysable tannins. The antimicrobial activity of phlorotannins was greater than the activity of both condensed and hydrolysable tannins found in terrestrial plant species.

Phlorotannins may also aid in decreasing methane emissions from ruminant animals by interfering with methanogenic microorganisms. While the ability for terrestrial tannins to reduce methane production has been well documented (Hess et al., 2005) the effect of phlorotannins on methanogenesis requires more research. Wang et al. (2007) investigated the effects of phlorotannins extracted from *A. nodosum* on the *in vitro* ruminal fermentation of alfalfa and barley silages and barley grain. Phlorotannin treatments were 0, 125, 250, and 500 µg/mL. It was found that treatments containing phlorotannins reduced total gas production in flasks containing both forages and barley grain substrates. Interestingly, total gas production was more negatively affected in forages than grains, suggesting that phlorotannins may be more detrimental to cellulolytic than amylolyitic bacteria. Methane production was measured in a second *in vitro* experiment, in which forage substrate was fermented with 0 or 500 µg/mL of phlorotannins. Methane production was significantly reduced over a 24 h period in the phlorotannin supplemented treatment. In their study, the reduction in methane was believed to be attributed to

the overall reduction in gas production and not necessarily a direct effect on methanogens. Further studies need to be conducted on the mechanism of pholorotannin action on ruminal microorganisms as well as the impacts of phlorotannins on methane production *in vivo*.

Antioxidant Activity

Due to their photosynthetic nature, plants including marine algae must be exposed to UV light in order to synthesize glucose. The UV light along with the oxygen by-product of photosynthesis can result in the production of damaging free radicals. Despite the prevalence of free radicals, plants appear to suffer minimally from oxidative stress, indicating the presence of protective compounds which guard the plants from damage (Heo et al., 2004; Li et al., 2011). Antioxidant activity of brown algae species including *A. nodosum* has been attributed to vitamins and minerals including vitamin E and selenium as well as to phenolic compounds such as phlorotannins (Allen et al., 2001). Antioxidant status of animals supplemented with *A. nodosum* has been examined in lambs subject to heat stress (Saker et al., 2004), goats subject to travel stress (Kannan et al., 2007), and steers grazed on endophyte infected tall fescue (*Festuca arundinacea*) pastures (Allen et al., 2001). These studies have produced varying results on the effectiveness of *A. nodosum* for minimizing damage by free radicals.

Cortisol is frequently used as an indicator of stress in animals. Plasma cortisol levels in lactating Jersey cows were examined in a study by Stelwagen et al. (1998) to investigate its influence on tight junction permeability within the mammary epithelium when cows were milked once or twice daily. Stelwagen et al. (1998) collected blood samples every 2 hours and found concentrations of cortisol in Jersey cows to be 4.6 ng/mL and 7.3 ng/mL for animals milked twice and once daily, respectively. A third treatment group of Jersey cows that were milked once daily were administered with adrenocorticotrophic at 5 time-points over a 10 hours period to

stimulate cortisol secretion, and the average cortisol concentration for this treatment group was 54.7 ng/mL.

West et al. (1991) examined the influence of bovine somatotropin in heat stressed Jersey and Holstein cows. The study took samples of milk and blood for analysis, including serum cortisol. Serum cortisol levels were unaffected by bovine somatotropin and were 9.96 and 8.13 ng/mL for treatment and control cows respectively. Unfortunately the study did not report cortisol concentrations of Jersey cows versus Holstein cows.

Cortisol is a hormone which response in times of acute stress, because of its rapid response cortisol concetrations vary greatly depending on time of day, environmental conditions, breed and stage of lactation. While a baseline level for plasma cortisol in Jersey cows is currently unpublished, these studies provide some evidence for cortisol concentrations in lactating cows.

In a study conducted by Allen et al. (2001) an extract of *A. nodosum* was applied to tall fescue pasture, which was infected with an endophyte that produces alkaloids that have been found to reduce animal performance and lower immune function. Over a 2 year period, 2 groups of 48 steers were assigned to treatments of endophyte or endophyte free tall fescue pasture with or without an extract of *A. nodosum*. Steers that grazed endophyte infected tall fescue pastures treated with *A. nodosum* showed increased phagocytic activity, suggesting that the extract of *A. nodosum* may have an immune system booster effect, which may negate the harmful effects of endophyte positive tall fescue.

Kannan et al. (2007) utilized 40 mature female meat bred goats in an experiment to examine the impact of *A. nodosum* on plasma cortisol, serum antioxidant status, and immune function when animals were subjected to transportation and pre-slaughter stress. Goats were assigned to 1 of 2 treatments: 1) control (0% KM), or 2) 2% KM of diet DM. Animals were fed

for 3 weeks prior to the 6-hour transportation test followed by overnight holding and fasting (to stimulate pre-slaughter conditions). Blood samples were taken at 0, 2, and 6 h of transport and after a 24 h fasting period in a holding pen. No significant difference was found in plasma cortisol between treatments. Lipid peroxidation was significantly lower in KM treated goats than in those fed the control treatment. Glutathione peroxidase activity was significantly higher in does treated with KM compared with the control diet. The decrease in lipid damage along with the increase in antioxidant activity by glutathione peroxidase indicate that *A. nodosum* may influence immune function by improving antioxidant activity and decreasing oxidative damage.

Saker et al. (2004) examined the immune response and antioxidant activity in 27 heat stressed lambs fed 3 different treatments: 1) control with no *A. nodosum*, 2) a diet in which hay was harvested from a field treated with an *A. nodosum* extracts (Tasco-Forage) or 3) a diet with non-treated hay supplemented with dried and ground *A. nodosum* at 1% of the diet. Treatments were fed for 3 weeks prior to the 2 heat stress periods. The heat stress period 1 consisted of moderate heat stress: 17 and 26°C night and day, respectively, for 4 d. The heat stress period 2 immediately followed period 1 and consisted of more significant heat stress: 21 and 31°C night and day, respectively, for 6 d. Blood samples were collected at the end of each heat stress period. Packed cell volume was analyzed to monitor hydration status, glutathione peroxidase and superoxide dismutase were analyzed as measures of antioxidant activity, and immune system function was evaluated by monitoring phagocytic activity and oxidative burst of monocytes. Control lambs had significantly more difficulty maintaining adequate hydration compared with the *A. nodosum* treatments. Lambs on treatment 3 (i.e., direct supplementation with *A. nodosum*) showed increased phagocytic ability compared with the other 2 treatments. This study indicates

that *A. nodosum* appears to improve the hydration status and immune function in lambs subject to heat stress conditions.

Estimation of Pasture Intake

Determination of pasture intake in grazing ruminants is challenging and can complicate pasture-based studies. Several methods have been examined for estimating pasture intake in grazing dairy cows. Two commonly used methods rely on the use of markers (internal or external) or on the estimation of herbage biomass pre- and post-grazing. It is important to note that direct measurements of pasture biomass pre- and post-grazing is best suited for estimating group intake rather than individual animal intake. Internal markers are indigestible compounds, which are naturally present in herbage. For instance, lignin, acid insoluble ash (**AIA**), and even-chain alkanes have been investigated as internal markers (Peyraud, 1997). External markers are indigestible compounds, which are not naturally occurring and must be supplemented to cows; chromium oxide and odd-chain alkanes are 2 of the most commonly used external markers (Peyraud, 1997).

Methodology for using chromium oxide to measure pasture intake requires the supplementing of chromium oxide to the cow for a minimum of 6 d prior to fecal collection (Peyraud, 1997). Methods of supplementation have varied by experiments but several common methods include dosing with time-release chromium boluses, dosing with chromium infused paper or incorporating chromium into concentrates and allowing the cow to consume the concentrate (Peyraud, 1997). Once fecal samples have been collected and analyzed for chromium content results may then be used to calculate fecal output. Herbage *in vitro* digestibility or another method of calculating herbage digestibility is necessary for the calculation of herbage intake based on the chromium methodology (Peyraud, 1997). In the case of n-alkanes, fecal output is

calculated by dosing the animals with a known concentration of synthetic odd-chain alkanes and comparing the fecal ratio of the odd-chain alkanes to the even-chain alkanes naturally present in the herbage consumed in the diet (Peyraud, 1997).

Ferreira et al. (2004) compared the effectiveness of n-alkane vs. chromium sesquioxide-AIA marker methods of estimating feed intake. In their study, markers (Cr_2O_3 and alkane $C_{31/32}$) were placed in time released boluses, which were then placed directly into the rumen via cannulas. A total of 8 non-lactating animals were utilized (n = 4 Holstein-Friesian cows and 4 Barrosa cows). In addition to the use of markers, the actual intake of animals was also recorded. It was found that the chromium-AIA method resulted in DMI estimates close to actual DMI but it was subjected to more variation compared with the n-alkane method. It is important to note that a large number of ruminally cannulated cows is logistically difficult to manage in pasture-based research. Therefore, direct rumen dosing of markers is not likely to be a widely adopted practice. However, Ferreira et al. (2004) indicated that time release markers can be effectively used to estimate pasture intake in cattle.

Macedo et al. (2010) showed that chromium oxide can be used as a reasonable indicator of fecal production and forage intake. Chromium oxide was used as an external marker and supplemented to steers at a rate of 10 g per day to provide 6.84 g of chromium. Fecal production calculations were compared to total fecal collections in 5 steers fitted with fecal collection bags. The mean for fecal production determined by *in vivo* fecal collection was 3.51 kg of DM/d while the mean of the morning and afternoon chromium sampling estimated fecal output at 3.54 kg of DM/d. Coefficients of variation were 11.8%, 18.0%, and 20.2% for total fecal output, chromium estimate from morning sample, and chromium estimate from afternoon sample, respectively.

These results indicate that fecal output estimates based on chromium as an external marker yields results similar to *in vivo* fecal collection, with only a slightly higher coefficient of variation.

Smit et al. (2005) compared 3 methods for estimating herbage intake in 12 lactating Holstein-Friesian cows during 2 grazing seasons. The 3 methods examined were pasture biomass calculations, n-alkanes, and calculations using net energy of concentrate and herbage with fat and protein corrected milk production and body weight (van Es, 1978). For the biomass method, cows were grazed in individual paddocks which were sampled by collecting and weighing 5% of the pasture area pre-grazing and 10% of the pasture area post-grazing. Herbage regrowth was accounted for by using herbage accumulation calculations with a light interception and use simulator for grasslands (Schapendonk et al., 1998). For the n-alkanes technique, animals were fed 1.5 kg of concentrate containing 0.3 g/kg of n-alkanes twice daily starting 7 d prior to fecal sample collection. It was found that the n-alkanes technique to be more consistent between years of study than the biomass method. The biomass technique had a higher coefficient of variation and was thought to underestimate pasture intake in year 1. The net energy method was found to be similar to n-alkanes and biomass calculations in year 1, but only a trend was found between net energy calculations and n-alkanes in year 2, with no relationship found between net energy and biomass. The study concluded that n-alkanes were the most reasonable method for estimating pasture intake because it remained the most consistent between years of study.

Studies have suggested that internal markers such as AIA or lignin may also be used to calculate fecal output and forage intake. Macedo et al. (2010) compared calculations using these internal markers vs. total fecal output using 5 steers. For both lignin and AIA the percent recoveries were greater than intake calculated based on feed content. Access to *ad libitum* mineral supplements in addition to forages can cause the percent recoveries to be higher than the

calculated intake. If additional mineral supplements are used, AIA is not a reasonable indicator of intake (Macedo et al., 2010).

Selecting a method for estimation of pasture intake is based on a wide variety of factors including time available, expense, labor, and pasture composition. All methods of estimating pasture intake have a certain degree of variation and are highly dependent on environmental and experiment consistency. Biomass measurements have the greatest success when pasture species is homogenous and the topography of the pasture is flat but sample collection may be affected by pasture collection methods, the individual doing the sampling and by varying pasture species (Peyraud, 1997). Markers such as chromium, AIA, and n-alkanes are better suited to determining individual herbage intakes. The accuracy of chromium markers can be affected by the quality of pasture samples taken for analysis of digestibility. Accuracy of n-alkanes can be impacted by the synthetic alkanes used and the levels of naturally occurring alkanes in pasture samples. With all marker methods pasture sampling must be representative and dosing of markers consistent (Peyraud, 1997).

Heat Stress

Although heat stress conditions are typically associated with dairy cattle raised in the Southern and Southwestern portions of the United States, mid-summer temperatures in the Northeast can rise above the critical upper limit suggested for dairy cattle. According to Berman et al. (1985) the critical upper temperature for dairy cows is 25 to 26°C. Temperatures above the critical upper limit are outside the animal's thermal neutral zone and result in the animal expending excess energy to keep cool. The shift in energy towards cooling may result in decreased animal performance and milk production. Ambient temperature, humidity. and solar radiation can all have an impact on the heat stress response in dairy animals (St. Pierre et al.,

2003) Therefore, the temperature humidity index is used as an indicator of climate conditions which may contribute to the heat stress of dairy cattle.

Previous studies have examined the potential for *A. nodosum* to mitigate the effects of heat stress on dairy cattle performance. In a study conducted at Kansas State University (Cvetkovic et al., 2004), 24 lactating Holstein cows were blocked by parity, DIM, and ECM. Cows were housed in a tie stall barn and ambient temperature and relative humidity were recorded daily. Control treatment consisted of no *A. nodosum* supplementation while the second treatment consisted of 113 g *A. nodosum*/d for 1 week followed by 57 g of *A. nodosum*/d for the remaining 5 weeks of the study. The *A. nodosum* used in this study was Tasco 14 made by Arcadian Seaplants. Rectal temperature, body surface temperature, and respiration rate were measured 3 times/d for 3 d of each week and were used to determine heat stress. Milk samples were collected twice weekly. No significant difference was found for any of the heat stress indicators examined, as well as for DMI. However, cows supplemented with *A. nodosum* did produce significantly more milk (35.2 kg/d) compared with non-supplemented cows (33.5 kg/d). There was no difference in milk fat percent or yields but there was a significant difference in milk protein yield between treatments with *A. nodosum* supplemented cows producing more milk protein (1.1 kg/d) than control cows (1.0 kg/d). There was also no significant difference for ECM or FCM. In this study, *A. nodosum* was unsuccessful in mitigating the effects of heat stress in dairy cattle, therefore the mechanisms by which milk and milk protein yield were increased was not related to a decrease in heat stress and must be the result of another mechanism by which *A. nodosum* impacts dairy cow performance.

In another study, *A. nodosum* was supplemented to dairy cows at the University of Missouri (Pompeu et al., 2011) utilizing 32 lactating Holsteins blocked by parity and DIM and

fed different levels of a commercially available dried and ground *A. nododum* product (Tasco). Cows remained on study for 3 periods with the first period used for adjustment to Calan doors and initial measurements, no treatments were fed during this time. Cows were assigned to 1 of 4 treatments in a randomized block design: control (0 g of Tasco), crossover (0 g of Tasco in period 2 followed by 115 g of Tasco in period, 3) 59 g of Tasco for both periods, and 4) 124 g of Tasco for both periods). Tasco was mixed with dry distillers and top dressed on TMR. Cows were housed in a free stall barn where temperature and relative humidity were recorded every 15 min using temperature loggers. Indicators of heat stress were measured 3 times daily and included skin temperature at the rump and ear, respiration rate, and core body temperature measured using a bolus inserted into the reticulum. Supplementing cows with Tasco had no significant effect on milk production, respiration rate or core body temperature. On the other hand, Tasco supplementation at a rate of 59 g/d reduced the skin surface temperature at the rump compared to the other treatments. Much of these findings are consistent with that of Cvetkovic et al. (2004) which also found no effect of *A. nodosum* on mitigating heat stress.

In these 2 studies examining the effect of *A. nodosum* on mitigating heat stress (Cvetkovic et al., 2004; Pompeu et al., 2011), the ambient temperature and humidity were not controlled by researchers and varied with environmental conditions. To better examine the question of the effects of *A. nodosum* on mitigating heat stress in dairy cattle, the environmental conditions should ideally be controlled by researchers to ensure that cattle are under heat stress during the measurement periods. However, this is not feasible in studies of grazing dairy cattle, therefore, close monitoring of environmental conditions including temperature, humidity and solar radiation is necessary in order to establish a relationship between *A. nodosum* and heat stress.

Milk Fatty Acids

The proportions of different fatty acids found in cow milk are important in the current study for two reasons; one because of their relevance to human health and two because of their ability to act as markers of rumen microbial populations. Saturated fats have been found to increase risk of obesity and heart disease while unsaturated fats may reduce these risks, therefore health professionals often advise humans consume diets lower in saturated fats and higher in unsaturated fats. Animal proteins including milk are characteristically high in saturated fats and there is heavy interest in manipulating animals' diets to lower these levels while increasing levels of unsaturated fats (Franklin et al., 1999). Saturated fats (12:0, 14:0 and 16:0) are of particular concern when it comes to increasing cholesterol in humans.

Previous studies have examined the use of fish oil and algae meal to manipulate milk fatty acid profiles of lactating dairy cows. Both fish oil and algae meal are high in long chain (n-3) fatty acids and may alter milk fatty acid profiles. Franklin et al. (1999) fed a rumen protected and rumen unprotected algae meal (*Schizochytrium* sp.) to lactating dairy cows and examined the impacts on milk fatty acid profile. This algae species was roughly 3.7% fat, about 1% higher than the KM used in the current study. Franklin et al. (1999) found lower DMI in algae supplemented animals, although there was no effect on milk yield, potentially indicating that algae supplemented cows were more efficient. Algae supplemented cows had significantly lower yield and percentage of milk fat. This study found lower levels of saturated fats and higher levels of unsaturated fats in supplemented cows. Algae feeding resulted in significantly higher levels of DHA and CLA. Cows consuming the protected algae had higher levels of DHA in their milk then cows consuming unprotected algae, indicating that fatty acids present in marine species may

be utilized by rumen microorganisms via biohydrogenation preventing them from being incorporated into the milk fatty acid profile.

In addition to being a concern for human nutrition and health, milk fatty acid concentrations have also been used as markers of the rumen environment and microbial populations. Odd and branched chain fatty acids are often considered as markers as they are found in low concentrations in feedstuffs but high concentrations in bacteria as components of bacterial membrane lipids. Although it is not yet possible to differentiate specific bacterial species based on milk fatty acid profile, examining levels of odd and branched chained fatty acids is still a viable technique for examining changes in ruminal microbial populations (Vlaeminck et al., 2006). Cellulolytic bacteria contain high amounts of *iso*-fatty acids, while amylolytic bacteria contain higher levels of linear odd chain fatty acids (Vlaeminck et al., 2006).

CHAPTER II

SUPPLEMENTATION OF INCREMENTAL LEVELS OF KELP MEAL TO LACTATING ORGANIC DAIRY COWS DURING THE WINTER SEASON

Introduction

Organic dairy farming is a rapidly growing segment of the agricultural industry with an estimated increase of 25% between the years 2000 and 2005 (McBride and Greene, 2009). Rules for organic agriculture production differ from conventional agriculture and are closely monitored by the USDA National Organic Program (**NOP**). All feeds, supplements, and animal health supplies must be organically certified. The use of antibiotics is prohibited on organic dairy farms, as well as the use of hormones, herbicides, pesticides, chemical fertilizers, and genetically modified crops. According to the NOP access to the pasture rule, organic systems must provide cattle with access to the outdoors year round and during the grazing season pasture must contribute to a minimum of 30% of the total DMI.

Despite growing popularity of organically certified products, there is a lack of scientific-based information examining various aspects of organic dairy farming. One of these aspects includes the use of nutritional supplements to lactating dairy cows. One commonly used mineral supplement in the Northeast is KM. Kelp meal is made primarily from the brown algae species *A. nodosum* harvested off the shores of the North Atlantic. After harvesting, the plant is dried, ground, and marketed as a mineral rich organic feed supplement for many livestock species as well as a mineral rich fertilizer.

Minimal scientific evidence is currently published to support the use of KM for dairy animals including lactating cows, dry cows, heifers, and calves. The following published research includes experiments where *A. nodosum* meal, extracts of *A. nodosum* or other kelp species were fed to livestock including dairy cattle (Berry and Turk, 1944; Cvetkovic et al., 2004; Kellog et al., 2005, Pompeu et al., 2011, Karatzia et al., 2012), calves (Erickson et al., 2012), beef cattle (Cradduck, 2001; Allen et al., 2001; Fike et al., 2001; Spiers et al., 2004; Bach et al., 2007;), goats (Kannan et al., 2007), sheep (Fike et al., 2001; Saker, 2004; Fike et al,, 2005; Bach et al., 2007; Archer et al., 2007), hogs (Gardiner et al., 2007), and in vitro (Wang et al., 2007; Wang et al., 2009).

Of the currently published studies in which KM was supplemented to dairy animals results have been varied. Cvetkovic et al. (2004) and Kellogg et al. (2006) reported that milk production was improved by KM supplementation in heat stressed dairy cows. However, Pompeu et al. (2011) found no significant effect of KM on milk production in heat stressed dairy cows. Cvetkovic et al. (2004) found no impact of KM on DMI. Berry and Turk (1944) fed a dried and ground supplement made from giant kelp and no impact of giant kelp meal on DMI. Pompeu et al. (2011) found a reduction in DMI with KM.

Several of the published studies on KM feeding examined the impacts of KM on blood profiles including but not limited to cortisol, glucose, and aldosterone and results of these studies have also been varied. Archer et al. (2005) observed lowered cortisol and aldosterone concentrations in lambs supplemented with KM and hypothesized that KM may be impacting the adrenal gland. Karatzia et al. (2012) found increased plasma glucose with KM feeding, potentially indicating a change in energy metabolism of supplemented animals.

Marine plants including the species fed in the current study (*A. nodosum*) contain high levels of iodine due to the high levels of iodine found in seawater (Bowman and Russell, 2006). The KM fed in the current study contained 820 mg/kg iodine (Table 2). In addition to marine plants and animals, dairy products also provide a dietary source of iodine to the human population as mammals concentrate iodine in milk to provide for the neonate (Bowman and Russell, 2006). Iodine is an essential mineral for mammals as it is a necessary component for the formation of thyroid hormones T_3 and T_4. In addition, iodine is necessary for reproductive function, growth and development and is essential for fetal neurocognitive development. (Bowman and Russell, 2006) Iodine deficiency is the leading cause of mental retardation worldwide and the reason for the implementation of iodized salt programs to prevent deficiency (Bowman and Russell, 2006) Iodine excess can also be detrimental to human health, resulting in hypothyroidism, hyperthyroidism, and goiter (Bowman and Russell, 2006). This is the first study to examine milk iodine concentrations of cows fed KM and aims to determine if the recommended levels of feeding KM result in milk iodine levels that may negatively impact human health.

Of the current studies available, none examine the impacts of KM during both the summer and winter seasons in the Northeast region of the United States. The majority of the currently published studies are conducted when animals are placed under stressful conditions such as heat stress, transport or exercise stress. The current study was conducted under routine organic dairy farm conditions over the winter months in the Northeast United States.

This chapter will investigate the feeding of incremental levels (0, 56, 113, and 170 g/d) of KM to organically certified lactating Jersey cows during the winter months (November to February) in which cows were fed a TMR composed primarily by mixed mostly grass and mixed

mostly legume baleages and a concentrate mash. No previous research was found where incremental levels of KM were fed to lactating dairy cattle. A dose-response study allows for determination of an optimum level of KM supplementation and if any negative consequences occur from feeding at the high rate of 170 g/d.

In addition to benefiting organic dairy farmers, this research is important to determine if the harvesting of *A. nodosum* for livestock feed is necessary. Kelp meal is produced from the ecologically important species *A. nodosum*, which provides a habitat and food source for a wide variety of marine life (Seeley and Schlesinger, 2012). If KM is not effective at improving dairy cattle health and performance, it may not be necessary to harvest this species as a feedstuff for livestock, thus reducing the risk of negatively impacting the rocky intertidal ecosystem.

The objective of this study was to determine if KM improves milk production and composition, nutrient utilization, and animal health as well as to find the optimal dose of KM per cow per day. It was hypothesized that KM would improve milk production and milk components as a result of additional vitamin and mineral supplementation, improved nutrient utilization, and feed efficiency. It was further hypothesized that animal health parameters will be impacted by KM, including reduced stress via reduced cortisol levels due to antioxidants present in KM and reduced plasma NEFA due to presence of vitamins capable of disrupting lipolysis including niacin. In addition, incremental feeding of KM may result in a linear increase of milk iodine due to high concentrations of iodine in KM.

Materials and Methods

Care and handling of the animals used in the current study were conducted as outlined in the guidelines of the University of New Hampshire Institutional Animal Care and Use Committee (IACUC Protocol # 111002).

Animals, Experimental Design, and Diets

Twelve multiparous Jersey cows averaging (mean ± SD) 40 ± 21 DIM and 464.4 ± 33 kg of BW and 4 primiparous Jersey cows averaging (mean ± SD) 75 ± 37 DIM and 384.5 ± 15 kg of BW were used in a 84-d long experiment conducted at the University of New Hampshire Burley-Demeritt Organic Dairy Research Farm (43°10'N, 70°99'W) from November 17th, 2011 to February 10th, 2012. Cows were blocked by parity, milk yield, and DIM and, within each block, assigned randomly to treatments in 4 replicated 4 × 4 Latin squares with treatment sequences balanced for carryover effects. The 4 dietary treatments consisted (as fed basis) of 0, 57, 113, and 170 g of KM (Thorvin Inc., New Castle, VA). Each period lasted 21 d with 14 d for diet adaptation and 7 d for data and sample collection. Cows were housed in a bedded pack barn (132 m^2) with dried pine shavings as bedding and had 24 h access to a cemented-floor pen (478 m^2) in order to comply with the USDA National Organic Program 'Pasture Rule" (USDA, 2010), which calls for year-round access to the outdoors for all ruminant animals.

The nutrient, mineral and vitamin composition of the TMR (which contained a 64:36 forage to concentrate ratio) is shown in Tables 1 and 2. The nutrient, mineral and vitamin composition of the concentrate mash (Morrison's Custom Feeds, Barnet, VT) used in the TMR is shown on Table 1 and 2. The forage component of the TMR consisted of first cutting baleages harvested from 2 different fields. Both fields were composed of cool season grass-legume mixtures with field 1 containing predominantly orchard grass (*Dactylis glomerata*) and field 2

containing predominantly alfalfa (*Medicago sativa*). Forages were cut using a conventional mower conditioner, tedded, and field-wilted to about 47.2% DM (field 1) and 49.9% DM (field 2). Forages were harvested as baleage using a large round baler with a crop cutter and wrapped with stretch plastic using a bale wrapper. The nutrient, mineral and vitamin compositions of baleages and KM used in this experiment are also shown in Tables 1 and 2.

Animal Feeding and Feed Sampling and Analyses

Baleage was weighed and chopped in a vertical mixer prior to adding the concentrate mash. After approximately 30 min mixing, the TMR batch was disposed on a cemented feeding deck, transferred to 121 L Rubbermaid trashcans, and weighed on a portable digital ground scale with 68 kg weight capacity before delivering to the cows after each milking. Individual intake was measured using the Calan door system (American Calan, Northwood, NH) system. Kelp meal was mixed with 454 g (as fed basis) of the same concentrate mash used in the TMR and offered to the cows in small rubber tubs immediately before their a.m. feeding to ensure complete consumption of the supplement. Cows assigned to the control diet (0 g of KM) received the concentrate mash (i.e., 454 g) only. Orts were collected daily prior to the morning feeding (approximately 0700 h) and weighed in the same manner as the TMR. The amount of feed offered to the cows was adjusted daily to yield refusals equal to approximately 5 to 10% of intake. Feed efficiency was computed by dividing mean milk yield by mean DMI while apparent efficiency of utilization of feed N was calculated by dividing mean milk N secretion (milk N concentration × milk yield) by mean N intake. Body weights were recorded at the same time for 3 consecutive days at the beginning of the experiment and at the end of each period to compute BW change. Animals had free access to water throughout the experiment.

All bales used in the current study were sampled prior to feeding using a battery-powered drill fitted with a metal core sampler (47 cm long). Throughout the duration of the study baleage samples (approximately 200 g each) obtained after 3 to 4 core samplings from individual bales were dried on microwaves with resulting DM used to adjust the daily amounts of TMR ingredients. During the sampling weeks, individual baleage samples collected daily were divided into 2 equal size (i.e., 100 g) subsamples. The first subsample was used for diet adjustments as described above while the second subsample was used to yield weekly composites by mixing equal amounts of DM of individual daily subsamples. Samples of TMR and orts were collected once a week throughout the experiment and daily during each7-d sampling week. The daily samples collected during each sampling week were composited by period. Concentrate samples were collected weekly and composited by period. A sample of KM was taken every time a 23-kg bag was open and then composited by period.

Samples of TMR, baleage, concentrate, KM, and orts were dried in a forced-air oven (55°C, 48 h), ground to pass through a 1-mm screen (Wiley mill; Arthur H. Thomas, Philadelphia, PA), and shipped to Dairy One Laboratories (Ithaca, NY) for wet chemistry analyses according to the following methods: DM (method 930.15; AOAC, 2006), total N (method 990.03; AOAC, 2006), NDF [Ankom Technology method 6 (NDF in feeds-filter bag technique for A200; Ankom Technology, Fairport, NY); solutions as in Van Soest et al., 1991], ADF [Ankom Technology method 5 (ADF in feeds-filter bag technique for A200; Ankom Technology); solutions as in AOAC method 973.18], crude fat (method 2003.05; AOAC, 2006), and ash (method 942.05; AOAC, 2006). The individual minerals (Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, Mo, and S) were analyzed using a Thermo IRIS Advantage HX or ICAP 6300 intrepid inductively coupled plasma radial spectrometer after microwave digestion (CEM application

note for acid digestion; CEM, Matthews, NC), while Cl ion was analyzed using a Brinkmann Metrohm 716 Titrino Titration Unit with silver electrode (Metrohm application bulletin no. 130; Metrohm Ltd., Herisau, Switzerland). Total mixed ration and KM samples were also analyzed for soluble protein (Cornell sodium borate-sodium phosphate buffer procedure; Cornell Nutrition Conference Proceedings, 1990), NDIN (LecoTruMac N Macro Determinator on NDF residue; Leco Corporation, St. Joseph, MI), ADIN (LecoTruMac N Macro Determinator on ADF residue; Leco Corporation), lignin [Ankom Technology method 9 (method for determining acid detergent lignin in the Daisy II incubator); solutions as in AOAC method 973.18], starch (YSI 2700 SELECT Biochemistry Analyzer; application note no. 319; YSI Inc. Life Sciences, Yellow Springs, OH), and ethanol soluble carbohydrates (Hall et al., 1999). In addition, TMR, baleages, concentrate, and KM samples were shipped to Agri-King Analab (Fulton, IL) for AIA analysis (Van Keulen and Young, 1977). Samples (TMR, baleages, concentrate mash, and KM) were also shipped to the University of Missouri-Experiment Station Chemical Laboratory for iodine analysis (AOAC 935.14, 2006). Samples of TMR and KM were further analyzed for fatty acids (**FA**) at Pennsylvania State University by gas chromatography after direct methylation (Sukhija and Palmquist, 1988). Kelp meal samples were additionally analyzed for AA (method 982.30; AOAC, 2006; University of Missouri Experiment Station Chemical Laboratories), B vitamins by HPLC (Dairy and Swine Research and Development Centre, Sherbrooke, QC, Canada), and Cr (Agr-King Analab). TMR, baleage, concentrate, and KM were additionally analyzed for iodine content by inductively coupled plasma mass spectrometry at Dartmouth College Trace Metal Analysis Laboratory (Hanover, NH).

Milk Sampling and Analyses

Cows were milked twice daily at 0500 h and 1530 h with milk yield recorded daily throughout the duration of the experiment. Milk samples were collected on d 6 and 7 (n = 4 consecutive milkings) of the sampling week and composited daily based on milk produced at each milking. Samples were preserved in tubes containing 2-bromo-2-nitropropan 1,3 diol, and kept at 4°C until shipped for determination of fat, protein, lactose, and MUN by mid-infrared reflectance spectroscopy (Dairy One Laboratories). Milk samples without preservative were frozen at -20°C and shipped to the Michigan State University Diagnostic Center for Population and Animal Health to be analyzed for iodine concentration following the method of Wahlen et al. (2005). Unpreserved milk samples from 2 (n = 8 cows) out of the 4 Latin squares were sent to the University of Vermont for FA analyses via gas chromatography.

Fecal and Urinary Sampling and Analyses

Fecal grab samples were collected from all 16 cows once daily on d 5, 6, and 7 of the sampling week at 0500 h, 1200 h, and 1500 by stimulating defecation or directly from the rectum via grab sampling. Samples were pooled by cow based on fresh weight (about 200 g per sampling) over the 3 d to obtain a single composite and then frozen at -20°C. At the end of each sampling week, composite fecal samples were thawed in aluminum trays and placed in a forced-air oven at 55°C until completely dried (approximately 72 h). Dried samples were ground to pass through a 1 mm screen (Willey mill) and analyzed for analytical DM, ash, total N, NDF, ADF, and AIA as described previously. Acid insoluble ash (Van Keulen and Young, 1977) was used as an external marker to estimate fecal output of DM (Cochran et al., 1986).

Spot urine samples were collected once daily on d 5, 6, and 7. Urine sampling occurred concomitantly with the fecal samples by stimulation of the pudendal nerve massaging the area

below the vulva. Immediately following each sampling, urine samples were combined with 800 µL of 6 *N* HCl, pooled by cow on a volume basis, and stored in 3 separate 50-mL centrifuge tubes at -20°C until analyses. After thawing at room temperature, urinary samples were analyzed colorimetrically for creatinine (assay kit no. 500701; Cayman Chemical Co.), allantoin (Chen et al., 1992), uric acid (assay kit no. 1045-225; Stanbio Laboratory, Boerne, TX), urea (diacetyl-monoxime method), and total N (micro-Kjeldahl; AOAC, 1990; Dairy One Cooperative Inc.). Daily urinary volume and excretion of total N were estimated from urinary creatinine concentration assuming a constant creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999). Urinary excretion of purine derivatives (**PD** = allantoin plus uric acid) was calculated based on the creatinine to PD ratio (Chizzotti et al., 2008) also assuming a constant creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999).

Blood Sampling and Analyses

Blood samples were taken once a day from the coccygeal vein on d 6 and 7 of the sampling week at 1200 h and 1500 h. After blood collection, EDTA tubes were kept on ice. Additive free tubes were not kept on ice and allowed to sit for at least 1 h prior to centrifuging. All blood tubes were centrifuged in an Eppendorf Centrifuge (5810R 15 amp) within 2 h of collection. Tubes were spun at 3,300 × g for 20 min at 5°C. After spinning, 0.5 mL of plasma from EDTA tubes was pipetted into 3 separate cryovial tubes and placed into the -80°C freezer. Plasma from additive free tubes was pipetted into 2 plastic test tubes (1 mL each) and placed into the -20 °C freezer. Blood samples collected in the second day were processed in the same manner and composited by cow on a volume basis with samples from the day before.

Serum samples were analyzed for thyroid hormones and cortisol. Plasma samples were analyzed for NEFA and urea. Thyroid hormone analysis occurred at Kansas State University

(Manhattan, KS) using the Siemens kit Cat# TKT4 for T_4 analysis and the Siemens kit Cat# TKT3 to analyze for T_3. Cortisol samples were run at the University of New Hampshire using the cortisol ELISA kit from BioVendor. Samples were run for NEFA concentration at the University of New Hampshire using a kit from Wako Pure Chemical Industries, (LTD HR Series NEFA-HR). Samples were run for urea concentration at the University of New Hampshire using a modified procedure from the University of Wisconsin based on the commercially available kit (Procedure No. 535, Sigma Chemical Comp).

Statistical Analyses

Animal-related variables were analyzed using the MIXED procedure of SAS (SAS version 9.3; SAS Inst. Inc., Cary, NC) according to a 4 × 4 Latin square design replicated 4 times. The following model was fitted for all variables:

$$Y_{ijkl} = \mu + S_i + P_j + C_{k(i)} + T_l + S \times T_{il} + E_{ijkl}$$

Y_{ijkl} = dependent variable, μ = overall mean, S_i = fixed effect of i^{th} square, P_j = fixed effect of j^{th} period, C_k = random effect of k^{th} cow within i^{th} square, T_l = fixed effect of l^{th} treatment (incremental dietary levels of kelp meal), $S \times T_{il}$ = interaction between i^{th} square and l^{th}, and E_{ijkl} = error term ~ N (0, σ^2_e). Orthogonal polynomials were used to test responses (linear, quadratic, cubic) from incremental dietary levels of KM supplementation. All reported values are least square mean and standard error of the mean. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$. The interaction and cubic terms were removed from the final model when $P \geq 0.25$.

Results

Kelp Meal Nutrient Composition

Along with the animal related variables examined in the current study, extensive analysis of the KM supplement was also conducted. In addition to the common nutrient analysis (Table 1) conducted for all feedstuffs, analysis of minerals and vitamins (Table 2), FA (Table 4), and amino acids (Table 5) was also conducted. As expected, concentration of iodine were higher in KM (820 mg/kg) compared to the TMR, which contained only 3.14 mg/kg of iodine. Kelp meal was hypothesized to be high in antioxidants including Se; however based on the analysis from the current study, Se concentrations in KM were < 0.41 mg/kg, and as a result the 170 g KM treatment provided approximately 0.07 mg of Se, which is much lower than the requirement for dairy cattle of 7.82 mg (Tisch, 2006). Kelp meal was relatively low in amino acids with the exception of glutamic acid (1.64 g/100 g) meaning that the 170 g treatment provided approximately 2.8 g of glutamic acid. The limiting amino acids for dairy cattle, methionine and lysine, were not found in high concentrations with methionine at 0.12 g/100 g and lysine at 0.32 g/100 g meaning with the highest treatment of KM only 0.2 and 0.55 g of methionine and lysine were provided, respectively. While KM was relatively low in crude fat (2.30%), FA analysis showed the most prominent FA to be *cis*-9 C18:1 (30.2 % of total FA), C16:0 (14.8% of total FA), and C14:0 (11.8% of total FA).

Animal Performance, Milk Composition, and Blood Metabolites

Milk yield, milk components, and measures of animal efficiency are presented in Table 6. No linear, quadratic or cubic effects were observed for milk yield. No effects were observed for milk fat, protein, lactose, solids non-fat, and total solids percentage or yield. There was also no effect of incremental levels of KM on MUN. There was no effect on BW or BW gain (Table 6).

Results of KM supplementation on serum hormone concentrations (T_3, T_4, and cortisol) and plasma metabolites (NEFA and urea) are found in Table 7. There was no impact of KM supplementation on thyroid hormone concentrations. A linear trend ($P = 0.08$) was found for decreasing serum cortisol concentrations with increasing KM supplementation. A linear relationship ($P = 0.05$) was found for decreasing plasma NEFA with increasing KM supplementation.

Milk Iodine

There was a positive linear relationship ($P < 0.001$) between KM supplementation and milk iodine concentration (Table 8). There was also a positive linear relationship for iodine intake with increasing KM supplementation. Apparent recovery of milk iodine decreased linearly with KM supplementation (Table 8).

Milk Fatty Acids

Milk FA responses are presented in Table 10. A quadratic response ($P = 0.03$) was observed for the FA C15:0 and a quadratic trend for milk FA C13:0 ($P = 0.10$). Linear responses were observed for the following milk FA: C7:0 (negative), *trans*-9 C14:1 (positive), *cis*-5, *cis*-8, *cis*-11, *cis*-14 C20:4 (negative), and *trans*-13 C22:1 (positive). Quadratic responses were observed for milk *trans*-4 C18:1, *cis*-11 C18:1, and *cis*-11 C20:1. There was no significant results for the remaining FA analyzed. There was no effect of treatment on the omega-6:omega-3 ratio or proportions of unsaturated FA.

Nutrient Intake and Apparent Digestibility of Nutrients

With a *P*-value of 0.09, a quadratic trend was observed between level of KM supplementation and DMI (Table 6). Dry matter intake peaked with the 57 g and 113 g KM treatments at 18.07 kg

and 18.06 kg respectively, while DMI was lower for the control and 170 g treatments at 17.52 and 17.62 kg/d, respectively. No differences were observed for OM, ADF, NDF or N intakes (Table 9). There were no differences observed for DM digestibility. A quadratic response was observed for ADF digestibility ($P = 0.04$) and quadratic trends observed for OM digestibility ($P = 0.08$) and NDF digestibility ($P = 0.10$) (Table 11). A negative linear trend ($P = 0.09$) was observed for N digestibility with increasing KM supplementation (Table 9).

Urinary Measures, N Excretion, and Estimated Bacterial Protein Synthesis

Results of this section are presented in Table 11. There were no significant effects of KM supplementation on urinary concentrations of creatinine, uric acid, allantoin, purine derivatives or urinary excretion of urea N (g/d, % N intake, and % urinary N). There was a significant quadratic response for urine N excretion (g/day) ($P = 0.03$) and a quadratic trend observed for urine N excretion % of N intake ($P = 0.10$). There was a significant quadratic response for PD/creatinine ratio ($P = 0.03$). There was a significant quadratic response for urinary concentration of allantoin ($P = 0.01$).

Discussion

Previous studies have exhibited contradicting results with KM supplementation and milk production. For instance, Cvetkovic et al. (2004) and Kellogg et al. (2006) reported that milk production was significantly improved by KM supplementation in heat stressed dairy cows. However, Pompeu et al. (2011) found no significant effect of KM on milk production in heat stressed dairy cows. Kellogg et al. (2006) conducted a study using 119 cows of varying breeds with cows blocked by size. The large breeds used were Holstein, Ayrshire, and Brown Swiss and the small breeds used were Jersey and Milking Shorthorn. Kellogg et al. (2006) found that milk

production improved when feeding KM, but there was an interaction with animal size. Smaller breed cows with lower milk production require lower levels of nutrients and, therefore, it is more likely that their needs were met prior to KM supplementation. Kelp meal may have had an effect on larger breed cows likely because not all nutritional needs were met by the diet alone. The lack of significant results in the current study may indicate that nutrient needs were met by the ration alone and, therefore, performance was not improved by additional mineral supplementation.

Even though a quadratic trend for DMI was observed for cows fed incremental amounts of KM, the actual differences in DMI between treatments were small and did not result in increased feed efficiency when expressed as milk yield:DMI. Cvetkovic et al. (2004) and Berry and Turk (1944) found no impact of KM on DMI. Pompeu et al. (2011) found a reduction in DMI when cows were supplemented with approximately 59 g/d of Tasco (a brown seaweed meal produced from *A. nodosum*); however, this reduction occurred only for a few days and no reduction was observed when supplementation was increased to approximately 120 g/d.

A study by Erickson et al. (2012) examined the hypothesis that high levels of glutamic acid in KM may lead to increase intake in dairy calves. However, this study reported that post-weaned heifers preferred the control diet (0 g of KM) rather than diets including 30 g or 60 g of KM, potentially indicating heifers did not find the KM to be palatable. High levels of glutamic acid have been associated with the umami taste and have been found to increase food palatability and intake in humans (Yamaguchi and Ninomiya, 2000). Glutamic acid levels of KM in the current study were 1.63 g/100 (Table 5), but it appears that this high concentration did not play a role in the quadratic trend observed for DMI in the current study. If glutamic acid would have been associated with DMI, a linear response would be expected.

An alternative hypothesis for the quadratic response observed for DMI is that at levels of 57 g to 113 g of KM resulted in improved digestibility, thus decreasing the time digesta spent in the rumen, reducing gut fill, and stimulating intake. At 170 g of KM it may no longer be beneficial to the ruminal environment and may limit digestibility, thus gut fill is not effected resulting in DMI similar to the control treatment. This study did find evidence to support this theory as quadratic trends were found for OM and NDF digestibility, and a significant quadratic response was found for ADF digestibility. Bendary et al. 2013 fed a seaweed product (Crossgates Biogenetics Seaweed, species not specified) to lactating dairy cattle and found significant improvements in digestibility of DM, OM, and CP and authors suggested that the improvement in digestibility may be due to the high levels of amino acids, FA, minerals, and vitamins found in KM. However, it remains unknown what component in KM may be responsible for influencing the rumen microbial populations leading to changes in digestibility.

Digestibility of ADF, OM, and NDF appeared to peak with the 57 and 113 g of KM and was lowest with the 170 g KM treatment. These results indicate that KM feeding may be beneficial to ruminal bacteria at lower levels of supplementation but may impair bacterial growth at levels exceeding 113 g. Based on these results, it is hypothesized that within the rumen KM may be beneficial to fiber digesting bacteria at levels of 57 and 113 g but harmful at levels of 170 g.

A negative linear trend was observed for CP digestibility ($P = 0.09$); similar CP digestibility was observed for 56.8 and 113 g treatments, but CP digestibility was reduced with the 170 g treatment. Therefore, it may be hypothesized that levels of KM exceeding 113 g/d could negatively impact other rumen microorganisms (proteolytic bacteria or protozoa) in addition to fiber digesting bacteria. Protozoa populations may be decreased as a result of less

fiber digesting bacteria as protozoa rely on fiber digesting bacteria as a source of N. An additional explanation for the decrease in CP digestibility could be due to the presence of phlorotannins in KM. Phlorotannins act like their terrestrial tannin counterparts and bind proteins and carbohydrates reducing their digestibility (Wang et al., 2009).

Fike et al. (2004) examined impacts of *A. nodosum* extracts (Tasco) supplementation on ruminal parameters in lambs under heat stress and thermal-neutral conditions. Three treatments were tested, a control diet, a diet containing hay treated with Tasco prior to harvest, and a diet including 1% Tasco over a period of heat stress and a period of thermo-neutral conditions. Results of this study found that Tasco improved both N and OM digestibility. This study also examined ruminal pH and VFA concentration and observed that butyrate concentrations were reduced with both forms of Tasco supplementation (10.3, 9.2 and 8.8 m*M* for control, treated hay, and direct supplementation, respectively). Total VFA and individual VFA concentrations (acetate, propionate, isobutyrate, and isovalerate) were not impacted by either form of Tasco feeding. The results of the Fike et al. (2004) study provide additional evidence that *A. nodosum*, or extracts of *A. nodosum* may be impacting the ruminal environment. While Fike et al. (2004) found N digestibility improved with feeding an extract of *A. nodosum*, rates of *A. nodosum* extract supplementation were 1% of diet DM, approximately 2.6 g/d. Lambs in the Fike et al. (2004) study lost weight over the course of the study and were experiencing a negative energy and N balance. As hypothesized in the current thesis, KM supplementation may be beneficial for nutrient deficient animals or animals in negative energy or N balance, whose needs are not met by their diet alone.

Previous studies have examined antimicrobial properties of phlorotannins isolated from *A. nodosum* both on in-vitro rumen microorganisms and on *E.coli* (Wang et al., 2008; Wang et

al., 2009). In Wang et al. (2008), phlorotannins reduced total gas production and aNDF and starch digestibility. A greater reduction in aNDF digestibility was observed when compared to starch digestibility, indicating that phlorotannins may be more detrimental against fiber digesting bacteria. Antimicrobial effects of *A. nodosum* were also observed in Gardiner et al. (2008) who examined the impact of KM feeding on intestinal bacteria in grower-finisher hogs. They showed that KM reduced ileal coliform and cecal *Bifidobacterium* counts in supplemented hogs. Thus, KM may be capable of influencing multiple species of microorganisms, potentially impacting nutrient digestibility.

In the current study the lactating Jersey cows exhibited a poor conversion of feed to milk. This poor feed efficiency may be attributed to the high forage composition of the diet. It is likely that the high NDF content of the diet resulted in cows having to consume large quantities of TMR in order to meet the demands for milk production leading to low feed efficiency across treatments. An additional explanation for the poor conversion of feed to milk may be provided by the environmental conditions in which cows were exposed. Cows had constant access to outdoors throughout the study with environmental temperatures ranging from a minimum of -20°C to a maximum of 17.7°C with the average temperature throughout the experiment of 0.33°C. Cold stress is known to increase energy requirements and DMI in cattle limiting the energy available for milk production (Fox and Tylutki, 1998). Cold stress is reached when temperatures are below the animal's lower critical temperature. Lower critical temperature is calculated by determining the heat produced by the animal and the animal's ability to insulate itself. Heat production by the animal is dependent upon energy retained and energy needed for pregnancy and lactation. The animal's ability to insulate itself is dependent upon body condition, wind velocity, hair coat, presence of mud, water or snow on coat, and hide thickness (Fox and

Tylutki, 1998). The barn design was setup so cattle must walk across an open concrete pad from a bedded pack barn to a separate feeding area. Because of this design, cattle were exposed to outdoors environmental conditions. As a result, there were times throughout the study where cattle were exposed to cold temperatures, moisture, and wind all of which may increase the lower critical temperature. Based on the Cornell Net Carbohydrate and Protein System model, maintenance requirement multipliers reached as high as 2.72 for heifers at temperatures of -20°C in high winds when animals were wet and matted, meaning that animals may need to consume as much as 2.72 times their maintenance requirements in order to maintain homeostasis (Fox and Tylutki, 1998).

Iodine exists in high concentrations in seawater which is taken up by marine organisms including *A. nodosum* (Bowman and Russell, 2006). *A. nodosum is* the predominant brown algae species which is dried and ground for the production of KM supplements. Previous studies have not examined the impact of KM on milk iodine concentrations; however, the correlation between other sources of dietary iodine and milk iodine has been well established (Franke et al., 2009; Castro et al., 2012).

In order for children to receive adequate calcium it is recommended they consume 4 cups of milk/d, which is the equivalent of approximately 1 L of milk/d (Pearse et al. 2004). The upper limit for iodine consumption in children ages 1 to 3 is 200 µg/day (Bowman and Russel, 2006). In the current study, milk iodine concentrations were 602, 1,015, and 1,370 µg/L for the KM treatments of 57 g, 113 g, and 170 g respectively. Therefore, children consuming the recommended 1 L of milk/d to receive adequate calcium intake would be over the recommended upper limit for iodine by a minimum of 402 µg/d with the lowest KM treatment (i.e., 57 g/d) and a maximum of 1,169 µg/d with the highest KM treatment (i.e., 170 g/d). While humans in

general tend to be highly tolerant of excess dietary iodine, it may result in hypothyroidism in some individuals, particularly those with underlying thyroid conditions (Bowman and Russel, 2006). In children, iodine in excess of 500 µg/d has been associated with decreased thyroid hormone production (Bowman and Russel, 2006), which can impair growth and neurocognitive development. Results of this study indicate that the addition of KM to diets of lactating dairy cattle should be closely monitored to avoid iodine excess in milk, which could result in adverse health conditions in children consuming recommended amounts of milk. The NRC (2001) lists the safe upper limit for iodine consumption by dairy cattle at 50 mg/kg. In the current study, iodine intakes from KM alone by dairy cattle were 47, 93, and 139 mg/kg for the 57, 113, and 170 g treatments, respectively. Therefore, supplementing KM to lactating dairy cows at levels of 113 and 170 g/d results in feeding iodine in excess of the NRC (2001) safe recommended limits. Levels of ethylenediamine dihydroiodine (EDDI) a common iodine supplement in the feed of dairy cattle has been limited to 10 mg/cow/day (8.03 mg I) to prevent iodine excess in milk for human consumption (Pearse et al., 2004). This type of regulation may need to be extended to KM products which this study has shown to be capable of linearly increase milk iodine.

Analysis of milk FA data showed that C15:0 was found to be lower in the 57 and 113 g treatments (1.17 and 1.13 %) and higher in the 0 g and 170 g treatments (1.20 and 1.18 %). Linear odd chain milk FA have previously been correlated with presence of amylolytic bacteria (Vlaeminck et al., 2006). As previously discussed, OM, NDF, and ADF digestibility were highest with the 57 and 113 g treatments. Therefore, decrease of C15:0 in milk of cows on 57 and 113 g treatments may further indicate a shift in the rumen microbial population with decreasing amylolytic species as cellulolytic species increased resulting in improved fiber digestibility. A quadratic trend ($P = 0.10$) was observed with the milk C13:0, another linear odd

chain FA with treatments of 57 and 113 g of KM resulting in the lower levels. However, presence of this FA was minimal (0.19% for the 57 and 113 g of KM and 0.20 for 0 and 170 g of KM) and the difference among treatments was very small; therefore, it is difficult to assess if this is truly due to a change in the ruminal environment.

Because cellulolytic bacteria contain high amounts of branched-chain FA these types of FA have been correlated with the increased presence of cellulolytic bacteria (Vlaeminck et al., 2006). Due to the increased fiber digestibility with the 57 and 113 g treatments, an increase could be expected in branched-chain milk FA. However, the current study showed no significant differences for *iso* C13:0, *iso* C15:0, *iso* C17:0, and *iso* C18:0. A quadratic response was observed for *iso* C14:0 ($P = 0.05$), with lowest levels occurring at 57 and 113 g treatments (0.15% of total milk FA) and slightly higher with 0 and 170 g treatments (0.16% of total milk FA). However, the overall proportion of this FA was minimal and the numerical difference among treatments was very small. Therefore, it unlikely this reflects any actual changes in the rumen microbial population.

Significant quadratic responses were also observed for milk *trans*-4 C18:1, *cis*-11 C18:1, and *cis*-11 C20:1; however, the levels at which these FA were observed in milk in the current study were extremely low. Statistically significant linear responses were observed for milk C7:0 (negative), *trans*-9 C14:1 (positive), *cis*-5, *cis*-8, *cis*-11, *cis*-14 C20:4 (negative), and *trans*-13 C22:1 (positive). Again, all of these FA were found in concentrations well below 1% of total milk FA and are likely not biologically important.

No significant difference was observed for the majority of measured FA in milk (Table 10). In addition, no differences were observed for total unsaturated FA or omega-6:omega-3 ratio. This indicates that feeding KM to lactating cows does not alter the milk FA profile in a

manner that would be beneficial to human health for example by lowering levels of saturated FA, increasing levels of omega 3 FA or increasing levels of conjugated linoleic acid.

Previous research has been conducted feeding other marine products [e.g., fish and other marine algae species (*Schizochytrium* sp.)] to lactating dairy cattle in attempts to alter FA profile. Interestingly, one of the most researched FA with supplemental feeding of fish and algae meals is C22:6 (n-3) commonly known as docosahexaenoic acid or DHA; however, this FA was not found in detectable levels in milk of cows on the current study. Previous studies have shown that in order to see a response in long chain milk FA, they must reach the abomasum of the animal (Franklin et al., 1999). In the current study, *cis*-9 C18:1 was found in high concentrations in KM, however, there was no difference in the concentrations of this FA across treatments. While the ability for ruminal microrganims to degrade KM is currently unknown, these results suggest that ruminal degradation is high, limiting the FA which reach the abomasum, thus limiting the presence of these FA in milk.

It appears that the current study is the first to examine milk FA profiles of lactating cows fed KM. However, Fike et al. (2004) examined the feeding of extracts of *A. nodosum* (Tasco) on meat FA composition via supplementing lambs with 3 treatments: control, Tasco treated hay, or direct Tasco feeding. These authors found a significant reduction in total saturated FA and a numerical increase in total unsaturated FA in meat of lambs supplemented with both forms of Tasco. Results of the Fike et al. (2004) indicate that FA metabolism in the ruminant may be impacted by *A. nodosum* supplementation.

There was no significant effect of incremental levels of KM on plasma T_3 or T_4 concentrations. Swanson et al. (1990) examined the impact of supplemental dietary iodine on thyroid hormone concentrations in lactating dairy cows and found no difference between

treatments. However, dietary iodine in Swanson et al. (1990) study was lower (74 mg I) than dietary iodine concentrations fed in treatments of 113 g (93 mg I) and 170 g (139 mg I) of KM. Convey et al. (1978) fed extremely high levels of dietary iodine 1,600 to 3,300 mg/d and found no difference between control and iodine supplemented cows. Therefore, it is not surprising that no difference in thyroid hormones was observed across treatments in the current study as a result of feeding KM.

There was a negative linear trend ($P = 0.08$) between increasing levels of KM and serum concentration of cortisol. Cortisol is a commonly used marker to indicate stress in animals. These results indicate that KM may help to mitigate stress in supplemented animals. Archer et al. (2005) measured cortisol concentration in lambs supplemented with incremental levels of Tasco (an *A. nodosum* based supplement) and found numerically lower cortisol concentrations in supplemented sheep during transportation with significantly lower cortisol concentrations at 4 h and a trend for lower cortisol levels at 8 h of transport. It was hypothesized in the current experiment that KM would reduce cortisol concentrations due to its vitamin and mineral content. Some vitamins and minerals found in KM including vitamin E, vitamin C, and Se have the ability to act as antioxidants, which can neutralize free radicals and lower oxidative stress. Selenium concentrations were analyzed and were lower than expected (<0.41 mg/kg; Table 2). Vitamin E levels were reported by Thorvin to be 110 mg/kg, and vitamin C levels at 375 mg/kg (personal communication: Ely Chandler). Antioxidant supplementation of vitamin C and vitamin E has been linked to decreased plasma cortisol concentrations and decreased heat stress response. In the study of Sivakumar et al. (2010), goats were subject to 3 treatments: control, 2 mg/d of vitamin C, or 250 mg/d of vitamin E. Goats were then exposed to heat stress with treatments of vitamin C and E resulting in significantly lower cortisol concentrations, body temperature, and

respiration rate when compared to control animals (Sivakumar et al., 2010). Goats on the Sivakumar et al. (2010) experiment received higher concentrations of antioxidants than those fed with KM in the current study.

Cradduck et al. (2001) examined *A. nodosum* as a source of Se for beef cattle, and examined whole blood Se concentrations after steers were grazed on pasture sprayed with Tasco-Forage (an *A. nodosum* based product) or a control pasture. Cradduck et al. (2001) found increased whole blood Se levels in steers grazing *A. nodosum* treated pastures on 2 of the 5 sampling days, indicating that *A. nodosum* may be capable of influencing the animal's antioxidant status.

Based on the reduction in cortisol concentration with increasing KM feeding in the current study, an additional hypothesis may be that KM is suppressing the adrenal glands. During the stress response, cortisol release is stimulated by adrenocorticotropic hormone which is released from the pituitary gland (Saladin, 2010). Cortisol falls into the category of glucocorticoids, hormones which regulate the metabolism of glucose. Cortisol regulates glucose metabolism by stimulating gluconeogenesis and the release and breakdown of fats and proteins (Saladin, 2010). If the adrenal gland is impaired by KM supplementation then cortisol would be produced and/or released in smaller concentrations potentially impacting gluconeogenesis and the breakdown of body fat. The hypothesis that KM feeding may impair adrenal function was proposed by Archer et al. (2005) who found lowered plasma cortisol and aldosterone concentrations in lambs supplemented with *A. nodosum*. Aldosterone is also secreted by the adrenal gland and regulates sodium and potassium balance in the body. Archer et al. (2005) also found lower IgG and IgM concentrations in lambs as KM supplementation increased. These results are concerning because they may indicate impairment of the animals immune response.

With an impaired immune system the animal is less capable of fighting off illness and infection. Lower immunoglobulin levels are additionally concerning in dairy animals as calves are born with no immune system and must receive all immunoglobulins from colostrum. The potential for KM to decrease IgG concentrations in colostrum should be examined in future research. Lowered IgG levels in colostrum may result in dairy heifers not achieving passive transfer, calves that do not achieve passive transfer have higher rates of mortality and are not as productive later in life.

In a second trial, Archer et al. (2005) examined the effects of 3 components within KM on plasma cortisol concentration in lambs including salt and the biologically active compounds fucoidan (a polysaccharide) and betaine (trimethylglycine) both found in high concentrations in KM (Archer et al., 2005). Of these treatments only the salt treatment resulted in lowered cortisol levels indicating that salt could be the compound within KM responsible for decreased adrenal gland function. However, the mechanism by which KM or the high concentrations of salt within it impairs adrenal gland function is currently unknown.

Plasma cortisol concentrations in the current study were substantially higher than cortisol concentrations reported by other studies (West et al, 1991; Stelwagen et al, 1998) The function of cortisol is to respond to acute stressors and the high cortisol levels observed in the current study could be a result of cow handling prior to and during blood sampling. Cows were restrained in headlocks for sampling which could have resulted in increased stress.

There was a significant negative linear relationship between increasing levels of KM and plasma concentration of NEFA. Circulating NEFA are indicators of lipid metabolism, with increased NEFA indicating an increase in fat mobilization from the adipose tissue. High concentrations of NEFA in the bloodstream can lead to metabolic conditions including fatty liver

and ketosis (Blowey, 1999). Reducing the concentrations of circulating NEFA is especially important in transition cows because they are highly susceptible metabolic diseases. These results indicate that KM may be effective in reducing fat mobilization from the adipose tissue. It was originally hypothesized that KM would reduce NEFA due to its high concentration of vitamins and minerals concentration, including niacin and chromium. However, after analysis of KM it was found that the niacin and chromium concentrations were not high enough to stimulate a reduction in FA metabolism when fed at the rates used in the current study.

An alternative hypothesis for the linear reduction in NEFA with increasing KM supplementation may be associated with plasma cortisol. Cortisol's role in the stress response in mammals is to increase the energy available to the body, particularly to the brain, in order to fight off the current stressor (Saladin, 2010). One of the mechanisms by which cortisol increases the energy available to the body is by increasing FA breakdown. The breakdown of FA is what results in increasing levels of NEFA in the bloodstream. If cortisol production is limited via disruption of normal adrenal gland behavior this could result in lower NEFA concentrations.

Further indications that KM may be influencing the ruminal environment may be demonstrated by examining the urinary excretion of allantoin and total PD (allantoin plus uric acid). A significant quadratic response was observed with the highest levels occurring with 57 and 113 g treatments and similar values for control and 170 g treatments (Table 11). *Ascophyllum nodosum* contains macro- and microminerals, vitamins, and phlorotannins that may exert stimulatory or inhibitory effects in the ruminal microorganims. Phlorotannins have been shown to exhibit in vitro antimicrobial activity against ruminal bacteria (Wang et al., 2008).

When urinary allantoin and total PD are compared to OM, ADF and NDF digestibility data a similar quadratic response is observed and may further indicate that the rumen microbial

population may be stimulated with levels of 57 and 113 g. However when KM supplementation is increased to 170 g allantoin, PD and digestibilities were similar to the control. Rates of KM supplementation over 113 g may negatively impact the rumen environment killing some microbial populations, lowering digestibility and decreasing microbial protein synthesis.

Urinary N excretion (g/d) had a significant quadratic response with the lowest N excretion occurring with the control treatment. This along with the negative linear response for N digestibility potentially indicates that KM supplementation results in changes in the ruminal environment limiting protein digestion. Phlorotannins present in KM may be forming complexes with proteins, limiting their digestibility and possibly the ability for the animal to absorb these complexes thus resulting in increased N excretion and decreased N digestibility with KM supplementation. Additionally a shift in ruminal microbial populations away from protein digesting microorganisms and towards cellolyticcellulolytic species may also result in decreased N digestibility and increased N excretion. In addition to limiting animal performance, decreased N digestibility and increased N excretion could result in increased N excretion to the environment.

Summary and Conclusions

The current study found no increase in milk yield or components with KM supplementation, however, rates of KM supplementation exceeding 113 g/d may cause milk iodine levels to become a concern to human health. Supplementation of KM did not result in a decrease in milk saturated FA or an increase in the milk FA associated with improved human health (CLA, DHA, omega-3 fatty acids).

Kelp meal supplementation at rates of 57 and 113 g/d appeared to improve digestibility of OM, ADF and NDF, although KM supplementation at 170 g/d lowered OM, ADF, and NDF digestibility's indicating that at high rates of feeding KM may negatively impact or alter ruminal microbial populations. This theory is supported by allantoin and PD results where a quadratic relationship was observed with highest concentrations occurring with 57 and 113 g treatments and concentrations with the 170 g treatment similar to that of the control treatment. Crude protein digestibility decreased linearly with KM supplementation and N excretion was increased further indicating a change in the ruminal population or a change in the digestibility of feedstuffs as a result of KM.

While KM supplementation did not directly improve milk production or milk component concentrations in lactating Jersey cows in the current study some potentially beneficial results on animal health were observed. Lowered plasma cortisol and NEFA concentrations were found with increasing levels of KM supplementation. These results may have positive implications including help to reduce incidence of ketosis and lower cortisol concentrations may indicate animals that consume KM are less stressed. However, if the reduction in cortisol is due to KM negatively impacting the adrenal gland this could be a greater concern than benefit. Prevention of disease is additionally important on organic dairy farms because the use of antibiotics is prohibited; therefore, if KM does impair immune function the effect could be detrimental on animal health and overall profitability.

Dairy farmers are paid on a yield and component basis and with no impact on milk production or component yields and with a cost approaching $2.20/kg, KM feeding is not likely to be a profitable economic decision for the dairy farmer. However, high organic grain costs are directing organic dairy farmers toward diets with low levels of concentrate supplementation or

all forage diets. In these cases, additional supplementation of minerals and vitamins will be necessary to meet the animal's demands and to avoid deficiency symptoms. Therefore, there may be a place in the organic dairy industry for KM supplementation.

In the current study the diet was balanced to meet all of the animal's nutrient demands therefore additional mineral supplementation via KM may be unnecessary thus did not result in improved animal performance. Without further research of how KM may impact animal immune function and without regulations on levels of KM feeding to prevent iodine excess in the human population, farmers should be cautious when feeding KM to lactating dairy cows, particularly at rates exceeding 113 g/d.

CHAPTER III

SUPPLEMENTATION OF KELP MEAL TO LACTATING COWS DURING THE NORTHEAST GRAZING SEASON

Introduction

According to the National Organic Program's (NOP) access to pasture rule, organic systems must provide cattle with access to the outdoors year round and during the grazing season pasture must contribute 30% of the daily DMI. Grazing season is specific to geographical location with the grazing season of the Northeast consisting of a minimum of 120 d. While on pasture, the impact of environmental conditions such as temperature, humidity, distance to pasture, and presence of parasites or predators can all influence the level of stress on the cow and, therefore, impact of stress on cow performance becomes of greater concern during the grazing season. When placed under stress the brain releases neurotransmitters which directly impact the milk ejection reflex. Under conditions of prolonged stress milk production in addition to the milk ejection reflex may be effected (Akers, 2002). Reducing stress to dairy cows on pasture can stabilize milk production during the grazing season, thus maximizing farm profitability. Stress in grazing cattle can come from numerous sources including those above mentioned, however heat stress is one of the most economically significant accounting for an average annual loss of $897 million in the dairy industry alone (St-Pierre et al., 2003).

Due to the seasons experienced in the Northeast, pasture is only an available forage source for dairy cows for approximately 6 months of the year. During the remaining months, the major forage source in the diet comes from stored forages made and preserved during the

warmer months. The impact of feed supplements including KM can vary greatly depending on season and what forage source is available for the cow. Therefore, it is important to examine the impacts of feed supplements both during the winter months when cows are consuming stored forages and during the warmer months when cows are consuming pasture.

Chapter II examined incremental feeding of KM to lactating dairy cows during the winter season. The current study aims to expand on that knowledge by examining KM feeding during the grazing season. Previous research examining KM feeding during the grazing season has focused at animals grazing endophyte infected Tall Fescue pastures, in attempts to learn if KM is effective at reducing the negative health and performance effects of grazing endophyte infected Tall Fescue (Allen et al. 2001; Fike et al, 2001). Previously published research on KM supplementation has not been focused in the Northeast therefore it is difficult to use these studies to determine how KM supplementation may influence grazing animals in the Northeast. Pastures in the Northeast are predominantly cool season grass species, forage species can have a large influence on how a supplement impacts an animal, and therefore it is important KM research be conducted here in the Northeast where KM feeding is common.

Based on the winter KM supplementation study, examined in Chapter II, 113 g of KM was selected as the dose rate of KM for the current study. This level of KM supplementation was selected to reduce the risk of potential harmful side effects in the human population due to excess iodine consumption. In addition, several significant and trend quadratic responses were observed for DMI and digestibility of NDF, ADF and OM indicating that 170 g of KM may be negatively impacting the ruminal environment.

The objective of the current study was to determine how feeding 113 g of KM impacts lactating dairy cattle during the grazing season in the Northeast, specifically examining milk and

component yields, milk iodine concentration, DM and nutrient intake, digestibility, and blood metabolites; including hormones (T_3, T_4 and cortisol) and indicators of energy status (glucose and NEFA).

It is hypothesized in the current study that the high levels of vitamins, minerals and other biologically active compounds including pholorotannins present in KM will result in increased milk yields and components including increased milk iodine, and lead to decreased cortisol and NEFA concentrations in plasma, when KM is supplemented at 113 g/day.

Materials and Methods

Care and handling of the animals used in the current study were conducted as outlined in the guidelines of the University of New Hampshire Institutional Animal Care and Use Committee (IACUC Protocol # 120504).

Animals, Experimental Design, and Treatments

Twenty organically managed lactating Jersey cows were housed at the University of New Hampshire Burley Demeritt Organic Dairy Research Farm. The study was conducted from June 13th 2012 to October 4th 2012 a total of 114 d. All cows were pure bred registered Jerseys, 8 multiparious cows averaged 163 (±44) DIM and 12 primiparious cows averaged 138 (±40) DIM at the start of the study. Multiparious cows averaged 441 (±28) kg BW and primiparious cows averaged 390 (±32) kg at the start of study. Cows were randomly assigned treatments of KM (113 g of KM) or control (0 g of KM), and remained on treatments for the duration of the study. For approximately 16 h/d cows were grazed on mixed mostly grass pasture. Average pasture DM was 25.7 % (nutrient analysis by period table 14). Pastures were managed using the strip grazing method in which the front fence was moved approximately every 12 h. Twice a day at 0400 h

and 1430 h cows were brought in from pasture and individually supplemented with treatments; 226.8g concentrate, 0 g kelp (control) and 226.8 g concentrate, 56.8 g kelp (KM treatment), using the Calan Door System. After consuming treatments, cows were milked in a four stall step-up parlor. A cholorhexadine based teat dip was used pre and post milking. After milking cows had access to a bedded pack barn, free choice water, and TMR fed using Calan doors (American Calan). The TMR consisted of 51% mixed mostly grass baleage (nutrient composition table 12), 47% concentrate blend (nutrient composition table 12) and .018% molasses. The concentrate blend was a cornmeal, barely based meal. TMR was fed to achieve 10% refusals. The TMR batch was mixed daily using a vertical mixer at approximately 1200 h, and each batch lasted 2 feedings. The TMR was adjusted daily to account for the DM of the baleage. At each feeding, TMR from the batch pile was weighed into trashcans using a floor scale (Rubbermaid Pelouze Digital Receiving Scale), and allocated to each cow individually using the Calan door System. Seven day sampling periods occurred every 3 weeks for 4 periods, with the exception of the fourth period which had a shortened 5 day sampling week to account for decreased pasture availability. During the shortened final period cows were on pasture only at night. To account for the decrease in pasture availability while cows were not on pasture they were provided additional TMR which contained a higher forage portion than the TMR fed in the first 3 periods and averaged 80% forage and 20% concentrate. This was done in order to keep the overall diet in the 4^{th} period as consistent as possible with the diet in the first 3 periods.

Feed Sampling and Analysis

Feed sampling of TMR and refusals occurred weekly during non-sampling periods and daily during sampling periods. Samples were collected by taking a representative handful from each of the 20 Calan doors. Samples were stored in zip-loc bags and frozen at -20°C. All samples

were thawed prior to drying which occurred in forced hot air ovens (Binder and VWR Scientific 1380) set at 55° C. Dry samples were then ground on a 1 mm screen using the Willey mill. Ground samples of the TMR and refusals collected during the sampling week were composited by period and shipped for analysis. Total mixed ration, baleage, concentrate, chromium pellet and KM samples were analyzed (Table 12) at Dairy One Laboratories (Ithaca, NY) for wet chemistry analyses according to the following methods: DM (method 930.15; AOAC, 2006), total N (method 990.03; AOAC, 2006), NDF [Ankom Technology method 6 (NDF in feeds-filter bag technique for A200; Ankom Technology, Fairport, NY); solutions as in Van Soest et al., 1991], ADF [Ankom Technology method 5 (ADF in feeds-filter bag technique for A200; Ankom Technology); solutions as in AOAC method 973.18], crude fat (method 2003.05; AOAC, 2006), and ash (method 942.05; AOAC, 2006). The individual minerals (Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, Mo, and S were analyzed using a Thermo IRIS Advantage HX or ICAP 6300 intrepid inductively coupled plasma radial spectrometer after microwave digestion (CEM application note for acid digestion; CEM, Matthews, NC), while Cl ion was analyzed using a Brinkmann Metrohm 716 Titrino Titration Unit with silver electrode (Metrohm application bulletin no. 130; Metrohm Ltd., Herisau, Switzerland), soluble protein (Cornell sodium borate-sodium phosphate buffer procedure; Cornell Nutrition Conference Proceedings, 1990), NDIN (LecoTruMac N Macro Determinator on NDF residue; Leco Corporation, St. Joseph, MI), ADIN (LecoTruMac N Macro Determinator on ADF residue; Leco Corporation), lignin [Ankom Technology method 9 (method for determining acid detergent lignin in the Daisy II incubator); solutions as in AOAC method 973.18], starch (YSI 2700 SELECT Biochemistry Analyzer; application note no. 319; YSI Inc. Life Sciences, Yellow Springs, OH), and ethanol soluble carbohydrates (Hall et al., 1999). Chromium were analyzed at Agri-King Laboratories and iodine content at University of

Missouri Experiment Station Chemical Laboratories (AOAC 935.14, 2006). Refusals were collected separately for each treatment prior to the afternoon feeding. After processing, refusals of each treatment were composited by period and shipped to DairyOne Laboratory for CP, ADF, NDF, and ash. Forages were harvested as baleage using a large round baler with a crop cutter (New Holland BR740A) and wrapped with stretch plastic using a bale wrapper (McHale 991BJS). All bales used in the current study were sampled prior to feeding using a battery-powered drill (HILTI model TE 7-A) fitted with a metal core sampler (47 cm long) Samples were stored, ground on 1 mm screen, and sampling week bales were composited by period prior to shipping for analyses. Concentrate was sampled once per period, samples were then dried, ground, composited by period, and shipped for analyses. Kelp meal samples were taken from each bag, samples were then composited and 1 sample shipped for the entire experiment. Liquid molasses was sampled per tub, composited into 1 sample, shipped and analyzed for CP, crude fat, ash, Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, Mo, and S, at DairyOne Laboratories.

Estimation of Pasture Intake

On day 17 of each period, 6 days before the start of each sampling week, cows were supplemented with 680.4 g of concentrate pellet (nutrient composition Table 12) containing an average of 5.4 g Cr, Cows on KM treatment continued to receive 56.8 g twice daily in addition to the pellet containing Cr. To account for the additional concentrate provided by the chromium pellet, 0.9 kg of concentrate/cow was taken out of the TMR. Chromium content of feces was used for estimating fecal output. In addition, 48 h in-vitro DM digestibility of pasture and TMR was used in the calculation shown below to estimate pasture intake.

Pasture intake = ((Cr fed/Cr fecal) – (DMI TMR × (1-IVDMD TMR))) / (1- IVDMD pasture) (Bargo et al. 2002)

Pasture Sampling and Analyses

During non-sampling weeks the twenty study cows were grazed with 18-20 other lactating Jersey cows. In sampling periods study cows were grazed separately from non-study cows to allow for pasture quality and pasture intake measurements. Pasture biomass was calculated pre and post grazing by tossing a 2.5 m^2 pasture square ten times per paddock. In each square pasture was cut to the ground weighed and dried. The area of each paddock was measured using a GPS. Pasture biomass were calculated using the method described in Bargo et al. (2012) method.

An additional 10 pasture samples were gathered using the pasture square and were used in determining pasture botanical composition; samples were separated into 4 categories grass, legumes, weeds and dead. For each paddock a representative pasture quality sample was dried and ground, paddock samples from each period were then composited and shipped for analysis of CP, ADICP, soluble protein, NDICP, ADF, lignin, NFC, starch, simple sugars, crude fat, ash, Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, Mo, S, Cl, and DCAD at DairyOne laboratories, using the above mentioned methods (Table 12)

Body Weights

Body weights were taken after the afternoon milking for 3 consecutive days before the beginning of the study and in the last 3 d of each period.

Fecal Sampling and Analyses

Fecal samples were collected 10 consecutive times (0500 h and 1500 h) during days 3, 4, 5, 6 and 7 of the sampling week. Fecal samples were composited by cow and immediately after collection were placed in aluminum trays and dried in a forced hot air oven at 55°C for a minimum of 72 h. Dried fecal samples were ground on a 1 mm screen and shipped to Dairy One Laboratories (Ithaca, NY) for wet chemistry analyses of DM, total N, NDF, ADF and ash (according to the methods referenced above). An additional 10 g were shipped to AgriKing for Cr analysis (method).

Milk Sampling and Analyses

Milk samples were collected for 4 consecutive milkings during days 1, 2 and 3 of the sampling week. Samples were preserved in tubes containing 2-bromo-2-nitropropan 1,3 diol, and kept at 4°C until shipped for determination of fat, protein, lactose, and MUN by mid-infrared reflectance spectroscopy (DairyOne Laboratories). Additional samples were shipped to University of Michigan for Iodine analysis using the method described in Wahlen et al., 2005. Milk samples were collected monthly by an independent individual from the Dairy Herd Improvement Association, these samples were analyzed for somatic cell count (SCC). Samples for SCC were collected by DHIA between the 13th and 15th of each month which directly followed the sampling week for each period. An initial SCC sample was taken on the start date of the study and a final SCC sample was taken 10 days after the conclusion of the study.

Blood Sampling and Analyses

Blood samples were collected via the coccygeal vein on days 1 and 2 of the sampling week. Blood was collected into EDTA tubes and additive free tubes to allow for normal clotting. Post collection EDTA tubes were stored on ice, additive free tubes were not. Additive free were

allowed to clot for 1 hour post collection. Tubes were spun in an Eppendorf Centerfuge (5810R 15 amp version) for 20 min at 3300 * g and 5 degrees C. 0.5 ml of serum per timepoint was composited and distributed into cryovials and stored in the -80 degree C freezer. One ml of plasma per timepoint was composted and distributed into vials and stored in the -20 degree C freezer. Serum samples were analyzed for cortisol and thyroid hormones. Plasma samples were analyzed for non-esterified fatty acids (NEFA), glucose, and urea. Plasma hormone analysis for T_3 (Siemens kit Cat# TKT3) and T_4 (Siemens kit Cat# TKT4) were run at Kansas State University. All other blood analysis were run at University of New Hampshire, Dairy Nutrition and Research Center in the following manner, NEFA (Wako Pure Chemical Industries, LTD HR Series NEFA-HR), urea (modified procedure from University of Wisconsin based on Sigma Blood Urea Nitrogen kit), glucose (kit # 510, Sigma Chemical Co., St. Louis), cortisol (BioVendor Cortisol ELISA kit).

Heat Stress

Measures of heat stress including respiration rate and rectal temperature were conducted during morning and afternoon milkings on 4 consecutive days on days 3-6 of the sampling week. Respiration rate was measured by counting the number of breathes per 20 seconds. Rectal temperature was taken using a rectal thermometer.

Statistical Analysis

The experimental design was a completely randomized block design. Cows were blocked by DIM, parity and milk yield. Data were analyzed with the Mixed Procedure of SAS (SAS version 9.3) for a completely randomized block design with repeated measures over time. The following model was fitted for all variables:

$$Y_{ijkl} = \mu + B_i + P_j + C_{k(i)} + T_l + P \times T_{jl} + B \times T_{il} + E_{ijkl}$$

where Y_{ijkl} = dependent variable, μ = overall mean, B_i = fixed effect of i^{th} block, P_j = fixed effect of j^{th} period, C_k = random effect of k^{th} cow within i^{th} block, T_l = fixed effect of l^{th} treatment, $P \times T_{jl}$ = interaction between j^{th} period and l^{th} treatment, $B \times T_{il}$ = interaction between i^{th} block and l^{th} treatment, and E_{ijklm} = error term $\sim N(0, \sigma^2_e)$. All reported values are least square means. Trends were declared at $0.05 < P \leq 0.10$. Interaction terms were removed from the final model when $P \geq 0.25$.

Results

Animal Performance, Milk Composition, and Blood Metabolites

Refer to table 16 for results. There was no observed treatment effect for milk yield. There was no effect of treatment on milk component percentages (protein, fat, lactose, solids non-fat, total solids). There were no effects on milk component yields of fat, total solids or solids non-fat. There was no effect on MUN. A treatment by period (T×P) interaction was observed for milk protein and lactose yields ($P = 0.02$) yet there was no treatment effect for these variables. No treatment effect were observed for any of the examined blood variables including T_3, T_4, cortisol, glucose, and NEFA. Although significant T×P interactions were observed for T_3 ($P < 0.001$) and T_4 ($P = 0.04$). No significant difference was observed for the indicators of heat stress (rectal temperature and respiratory rate) during both morning (A.M) and afternoon (P.M) measurements.

Milk Iodine

Refer to table 16. A significant treatment effect ($P < 0.001$) and T×P interaction ($P < 0.001$) was observed for milk iodine. Milk iodine levels were higher in KM supplemented

animals (592.1 versus 138.2 µg/L) Milk iodine levels were higher for both treatments in period 4 when compared to the first 3 periods.

Nutrient Intake and Apparent Digestibility of Nutrients

Refer to table 17. There was no treatment effect on TMR intake. A trend was observed for pasture intake ($P = 0.06$) with KM supplemented cows having increased pasture intakes over control cows (9.02 and 7.95 kg/day respectively). No treatment effects were observed for intakes of OM, N, ADF, and NDF. There were also no treatment effect of KM supplementation on digestibility of DM, OM, ADF, and NDF. Although a trend for T×P interaction ($P = 0.06$) was observed for OM digestibility with reduced OM digestibility for both treatments in Period 4. Additionally a trend towards a treatment effect ($P = 0.10$) was observed for decreased CP digestibility with KM supplementation (64.61% for control cows and 63.51 % of KM supplemented cows).

Pasture Allowance, Biomass and Botanical Composition

Based on pre-grazing biomass calculations outlined in the materials and methods, pasture allowance averaged 12.8 kg/cow/day. Pasture allowance was highest (21.09 kg/cow/d) in the first period (July) and lowest (7.84 kg/cow/d) in the second period (August). The area available to cows averaged 72.07 m^2/cow/d. Pregrazing herbage mass averaged 1608 kg/DM/ha and post-grazing herbage mass averaged 990 kg/DM/ha. Botanical composition of pasture averaged 57.28% grass (with orchardgrass as the predominant species), 16.51% legume (with white clover as the predominant species), 11.56 % weeds and 13.27% dead.

Discussion

Milk data from the current study (Table 16) agrees with data from the winter KM supplementation study (Table 6) indicating that KM is not effective at increasing milk yield, or milk components, nor did it result in improved animal efficiency; because dairy farmers are currently paid on a yield and component basis this indicates that KM will not help to increase overall farm profitability. Other studiers including Karatzia et al. (2012) and Pompeu et al. (2011) also found no difference in milk or component yields.

Although SCC was not statistically analyzed the results are explained below and shown in Figure 1. The initial SCC sample was taken on the start date of the study where SCC was (241 and 244 for control and KM treated animals respectfully) A final SCC sample taken 10 days after the end of the study was 100 and 109 for control and KM animals respectful. During the initial and final samples when animals were not on treatments SCC appeared to be relatively equal. However, during months (July, August, September) cows were on study the SCC of KM supplemented cows appeared to be lower than the SCC of control animals (Figure 1). Lower SCC is beneficial to the farmer because it corresponds to an improvement in milk quality. This can result in economic benefits for the farmer, who may receive more money for higher quality milk.

Milk iodine was significantly higher in KM supplemented cows (Table 16), corroborating results from the winter KM supplementation study. As previously discussed, excess iodine in milk is particularly concerning because it may negatively impact human health. Milk and other dairy products are an important source of iodine for the human population. Mammals including cows readily secrete iodine in milk to provide for the neonate, as iodine plays a valuable role in neurological development (Bowman and Russell, 2006). Throughout recent years human

nutrition has focused more on the impacts of iodine deficiency rather than iodine excess. Iodine deficiency is the leading cause of mental retardation worldwide which provides much of the motivation for iodine supplementation to the human population via iodized salt (Bowman and Russell, 2006). Iodine excess, however, has received less publicity and majority of the population has been found to be tolerant to high levels of iodine intake. However, iodine excess may be a concern particularly for infants and children as well as those with underlying thyroid conditions.

Milk iodine levels in both control and KM was increased during period 4 when cows were only allotted half as much time on pasture as the other 3 periods. These results coincided with several other studies which have shown a difference in milk iodine concentrations between summer and winter months (Pennington et al. 1990; Dahl et al., 2003; Pearce et al. 2004). Previous studies hypothesized that the increase in milk iodine during winter months was due to increased supplementation of TMR including iodine rich vitamin and mineral mixes. This is unlikely to be the reason for the change in milk iodine in the current study because feed iodine levels were too low to result in the milk iodine increase observed in period 4 (Table 12. Therefore, it is likely that the difference in milk iodine concentrations may be attributed to a compound found within the pasture which may inhibit iodine uptake by the cows or limit iodine secretion in milk. Goitrogen compounds are known to be found in legumes, including White Clover. White Clover contains hydrocyanic acid, which when consumed by the animals is converted to thiocyanate which is a goitrogen. (Frame et al. 1998). The botanical composition of pastures grazed in the current study averaged 16.5% legume with white clover as the predominant legume species present. Levels of hydrocyanic acid vary greatly depending on cultivar, plant age, moisture stress, grazing pressure and soil nutrient supply. (Frame et al. 1998)

Unfortunately goitrogens are highly sensitive to drying and pasture samples from the current study were dried and ground prior to analysis therefore samples were not analyzed for goitrogens.

No significant difference was observed for TMR DMI, however a trend was observed for increased pasture intake with KM supplementation (Table 17). During the winter supplementation study a quadratic trend (*P*-value = 0.09) was observed for DMI with the highest intakes for 57 and 113 g KM treatments. It is possible KM supplementation increased pasture intake due to a reduction in heat stress thus allowing them to consume more pasture. Spiers et al. (2004) found that supplementation of Tasco (*Ascophyllum nodosum*) reduced core body temperature and respiration rate in heat stressed animals.

Despite KM supplemented cows consuming more pasture than control animals this did not correlate with an increase in milk production or feed efficiency expressed as ECM:DMI or FCM:DMI. Cvetkovic et al. (2004) found an increase in milk yield and milk protein when animals were supplemented with KM yet had no differences in intake, resulting in improved feed efficiency. Therefore, feeding KM to lactating cows on pasture does not appear to be a viable option for farmers looking to reduce cost through improved feed efficiency or through increased milk or component yields.

There were no significant differences in nutrient intake across treatments. In the winter experiment there were also no significant differences in nutrient intake of OM, N, ADF, and NDF. The current study found no significant differences for DM, OM, ADF, and NDF digestibility, however, there was a trend for decreased CP digestibility in cows supplemented with KM. Similar results were seen in the winter KM supplementation study, where a linear trend was observed for decreased CP digestibility with KM supplementation. As hypothesized in

the winter KM study this effect could be the result of a shift in rumen microbial populations away from protein digesting microbes, although N excretion data is not available from the current study to verify these findings. Also hypothesized earlier is that the presence of phlorotannin compounds in KM may form complexes with proteins resulting in decreased digestibility.

Fike et al. (2005) found improved N digestibility when lambs were fed an *Ascophyllum nodosum* product (Tasco); however, in Fike et al. (2005) study lambs were limit fed and overall diet digestibility was poor, indicating that the use of KM may only be beneficial in animals consuming poor quality or mineral deficient diets and/or experiencing a negative energy balance.

During the winter kelp study cortisol levels were found to linearly decrease in cows supplemented with increasing levels of KM (Table 7). In the current study, cortisol levels were numerically higher for cows supplemented with KM during all 4 periods, although no treatment effect was observed ($P = 0.33$). Overall, cortisol levels were higher in the pasture study then the winter study. During the winter study, cortisol levels across all treatments averaged 66 ng/mL, while during the summer study cortisol levels were increased and averaged 103.4 ng/mL (Table 16) indicating cows were under more stress during the summer. This is likely due to a number of factors including heat stress, increased stress due to flies and increased stress due to more exercise including walking to and from pasture as well as walking around on pasture to graze. It is possible that KM was not effective at reducing cortisol when the stress on the animal is too great. Cradduck (2001) found Tasco had no effect on whole blood Se concentrations in stressed heifers, and suggested that potential antioxidant or immune benefits of *A. nodosum* may be compromised when animals are under stressful circumstances.

Plasma non-esterified fatty acid levels were similar between treatments in the current study (Table 16), yet were different in the winter study (Table 7). However average NEFA concentrations during the summer study were 116 µg/dL which was lower than the winter study were levels averaged 143.7. Cows on the winter study were earlier in lactation then cows on the summer study, 40 DIM vs 140 DIM respectively. Therefore the increased NEFA's observed during the winter experiment were likely due to cows experiencing more of a negative energy balance during this time resulting in increased fat mobilization. During the summer experiment cows were past peak lactation and not experiencing as much of a negative energy balance thus decreasing the need for the animal to mobilize body fat reserves. Environmental temperatures also varied greatly between the 2 experiments during the winter experiment cows were experiencing cold stress while during the summer experiment cows were experiencing heat stress. Therefore, KM may only be effective at reducing NEFA when cows are in early lactation or only when cows are experiencing cold conditions. Although lower NEFA levels may reduce the animals risk of ketosis, this may be concerning during the winter season as it could indicate the animal is less capable of mobilizing body fat to produce heat. Cows that are exposed to temperatures below their thermal neutral zone that are unable to mobilize body fat for warmth may be at risk of hypothermia and frost bite.

There was no difference in serum T_4 concentrations across treatments. Although there was no difference in T_3 levels for the overall experiment, a treatment by period interaction was observed. Serum T_3 levels are relatively equal during periods 1 and 2 of the study but are lower for KM fed animals during periods 3 and 4. This may indicate that over time KM feeding negatively impacts the production of T_3. While in the winter study no differences were observed between treatments it was a short term study with treatments changing every 21 d and the entire

studying lasting 84 d, while animals on the current study remained on their treatments for the duration of the 114 d. Within the animal, thyroid hormones are essential for maintain homeostasis and regulate essential functions including respiration rate, body temperature, and heart rate all of which may impact intake and milk production (Saladin, 2010). Lowered T_3 levels may result in a decrease in these essential body functions which could be an indicator of improved animal efficiency. Spiers et al. (2004) observed a reduction in core body temperature in animals supplemented with 1% Tasco and hypothesized that this reduction in core temperature may be due to a decrease in thyroid hormone levels thus reducing the signal to increase metabolic rate. Lower thyroid hormones levels may be an indicator of improved animal efficiency, however the current study found no difference in ECM:DMI or FCM:DMI.

There was no difference in plasma glucose concentrations across treatments, indicating that KM was not effective at improving nutrient utilization by the animal. Karatzia et al. (2012) found plasma glucose levels to be increased in animals fed *A. nodosum.* Cows used on the Karatzia et al. (2012) study were Holsteins producing an average of 34 kg of milk per day almost double the cows on the current study. Therefore KM may be more beneficial when animals are in a negative energy balance for example in early lactation cows producing high quantities of milk.

No difference was observed for indicators of heat stress across treatments. Both A.M. and P.M. rectal temperature and respiratory rate were similar across treatments for all four periods of study. These findings are consistent with Cvetkovic et al., 2004 and Pompeu et al., 2011 which also observed no significant effect on KM on mitigating heat stress. Although Cvetkovic et al., 2004 observed a significant difference was observed for milk production which was not seen in the current study.

Summary and Conclusion

Supplementation of KM to lactating cows over the grazing season in New Hampshire had no impact on milk yield, milk components. The current study also found KM supplementation to increase pasture intake in lactating cows, but found no difference in TMR intake, the increased pasture intake did not result in improved animal efficiency, indicating that KM supplementation is unlikely to result in increased profit for dairy farmers. A trend was observed for decreased N digestibility with KM supplementation, this is in agreement with the results of the winter study where N digestibility linearly decreased with increasing KM supplementation. *Ascophyllum nodosum*, the brown algae species used in KM production has been found to contain high levels of phlorotannins, biologically active compounds with similar activity to terrestrial tannins. These compounds have been found to form complexes with proteins limiting digestibility, and could be the cause for the observed results.

The major finding of the current study was the high levels of milk iodine found with KM supplementation. In agreement with several other studies (Pennington et al., 1990; Dahl et al., 2003; Pearce et al., 2004) milk iodine levels were lower during summer months when animals were consuming more pasture and increased when pasture intake was decreased. This is hypothesized to be due to the presence of goitrogen forming compounds present in pasture species including White Clover. Future research should be conducted to confirm the presence of goitrogen forming compounds in Northeast pasture species, and to determine if additional iodine should be supplemented to lactating cows grazing these species. Based on the results of the current study it appears that during the pasture season KM supplementation to lactating cows is not as great of a concern to human health via excessive milk iodine levels. However precautions should still be taken to avoid excessive milk iodine levels including increased recommendations

and guidelines becoming available to dairy farmers on acceptable levels of KM feeding. As with supplementation of EDDI a maximum level of KM supplementation should be set to avoid iodine toxicity issues in the human population.

Contradictory to the previously conducted winter study KM had no significant effect on cortisol possibly that any benefit of KM supplementation was not observed due to the elevated cortisol levels observed on the current study. There was also no effect on NEFA concentrations possibly due to cows being later in lactation then cows on the winter study. Therefore, KM supplementation may not be beneficial at reducing stress in lactating cows on pasture. Although not statistically proven the current study observed lower SCC in cows fed KM. The current study found no improved animal performance observed with KM supplementation future research is required to replicate these results and determine if KM feeding is recommended for late lactation cows on pasture.

CHAPTER IV

CONCLUSION AND FINAL REMARKS

The current studies found evidence to support that KM does indeed influence lactating dairy cattle. Perhaps the most prominent response being the impact of KM on milk iodine concentrations. Results from both studies indicate an increase in milk iodine when animals were supplemented with KM. Milk iodine in cows supplemented with KM may be elevated to levels that would be of concern to human health particularly for children and those with underlying thyroid conditions. Because of the potential risk to human health due to high iodine concentrations in KM increased feeding recommendations should be made for KM. Feeding recommendations are in place for other iodine mineral supplements including EDDI to prevent levels of iodine in milk from exceeding those recommended for human consumption.

The studies conducted in the present thesis suggest that KM supplementation had additional significant effects that should be validated with more research. Significant results from the winter study included a linear decrease in NEFA with incremental feeding of KM in the winter study, a quadratic response for allantoin (mM/d) and purine deriviatives (mM/d), and a quadratic response for N excretion. Several significant effects were observed in the analysis of milk fatty acids from the winter study, while some of the fatty acids that showed significant results were found only in very low concentrations possibly influencing results, a significant quadratic effect was observed for C15:0 which was found in a higher concentrations.

Significant results from the pasture kelp supplementation study, in addition to milk iodine concentration, included treatment by period effects for milk protein and lactose yields as well as

plasma T_3 and T_4 concentrations. Trends from the pasture study included a treatment effect for pasture intake and a treatment by period effect for MUN.

Future studies should take into consideration several factors from the current study to correct for potential problems and gain a better understanding of how KM supplementation impacts the lactating dairy cow.

Plasma cortisol concentrations found in cows in the current studies indicate cows under heavy stress likely due to an acute response from restraint and the blood sampling procedures. These high cortisol levels may not be an accurate reflection of the lactating dairy cow on a daily basis and therefore may not provide an accurate picture of how KM is influencing the lactating dairy cow. To avoid accurate cortisol responses due to handling procedures, utilization of fecal cortisol concentration in future studies may provide a more accurate depiction of how KM influences cortisol concentrations. While plasma cortisol is rapidly influenced under times of acute stress, fecal cortisol remains stable and may provide a more reliable estimate of how KM influences stress in the lactating dairy cow on a daily basis.

Measures of heat stress (respiration rate and rectal temperature) conducted in the pasture based experiment in the present thesis were taken twice daily regardless of environmental temperature. Environmental temperature was not under the control of the experimenters so there were times when environmental conditions were over the cow's thermal neutral zone and times when environmental conditions were not outside the cow's thermal neutral zone and cows were not experiencing heat stress. Therefore it is difficult to determine from this data the impact of KM on mitigating heat stress in the lactating dairy cow. To gain a better understanding of how KM may influence measures of heat stress housing cattle in a manner where environmental

conditions can be controlled by the experimenters would be beneficial. This however becomes a challenge as with the current experiment where animals are outdoors on pasture. Measurements of heat stress could potentially be taken only on days when conditions are outside of the cow's thermal neutral zone, thus allowing animals to remain on pasture while still gathering data of animals under heat stress.

The current studies relied on several techniques that allow for the estimation of volatile fatty acids, bacterial protein synthesis and pasture intake. There are several assumptions that must be made and issues which can arise when relying on such calculations.

One of the largest assumptions in the current thesis was that calculations based off data from Holstein cattle may be utilized for the Jersey breed. For example the equation for estimation of urine output is essential for calculating bacterial protein synthesis using the purine derivatives and is based off Holstein data. How well the urine output of Jersey cattle fits this model is currently unknown. Therefore the estimations of bacterial protein synthesis could be skewed if Jersey cattle do not properly fit the model. The future of dairy cattle research using the Jersey breed would greatly benefit from experiments conducted to determine the accuracy of these equations when Jersey cattle are used. Jersey cattle are often utilized on organic dairy operations and with the growth of organic dairy farming and increased demand for organic dairy based research the need to validate the use of these models for the Jersey breed cannot be under stated.

Any time calculations are used for estimations of experimental parameters a degree of uncertainty may be assumed. The use of ruminally cannulated cows would provide accurate physical information of how KM may be influencing the rumen environment. While the current

experiments used purine derivatives and milk fatty acids to estimate volatile fatty acids and bacterial protein synthesis, utilizing cannulated animals would allow researchers to take samples directly from the rumen for analysis of volatile fatty acids, bacterial protein synthesis and bacterial population proportions, thus removing some of the uncertainty from the data set. However the use of ruminally cannulated cows would not be humane on an organic dairy farm where access to antibiotics and other medications is restricted. Kelp meal research using ruminally cannulated cows could take place on a conventional research facility or rumen samples may be taken from organic animals using an esophageal tube.

The estimation of pasture intake also utilized calculations based on levels of chromium feeding and fecal output of chromium. Again assumptions must be made with these calculations, for example that chromium is fully consumed and is totally indigestible. As discussed in Chapter I (Literature Review) there are many methods of estimating pasture intake.

There are many directions in which the future of KM supplementation to dairy cattle may be taken. As with all research the current experiments should be repeated as to validate their findings. Aspects discussed in Chapter IV of the present thesis should be taken into consideration to better improve upon research methods and data analysis of KM supplementation to lactating cows in the future. The results of the current experiments raised some additional hypotheses that should be tested in future experiments and there are many experimental parameters that could be studied in greater detail. Some aspects which should be examined in future research include the potential of KM to influence adrenal gland function and the rumen environment. In addition the impact of KM supplementation on SCC should be analyzed in greater detail, a potential impact was observed in the pasture study but statistical analysis was not completed.

Future research in KM supplementation should focus not only on lactating dairy cattle but calves, heifers and dry cows as well. Of the 145 farms (64% of total farms) which reported feeding KM in some manner, 60% were feeding KM to calves, 82% were feeding KM to heifers and 82% were feeding KM to dry cows. Therefore the need to determine the impact of KM supplementation on these animals is also great.

In conclusion, results of previous research as well as results of the current two experiments indicate a need for further research in the area of KM supplementation. Based on the high use of KM on Northeast organic dairy operations, anecdotal evidence from farmers and significant differences observed in the current studies KM does indeed appear to be influencing milk quality as well as the health and performance of the dairy cow. Only future research will be able to verify the results of the current study and determine the mechanism of KM action in the dairy cow. Of utmost importance however is determining the potential impact of KM supplementation on the human food supply and addressing the potential need for regulations on levels of KM supplementation to dairy animals to prevent levels of iodine in milk from reaching concentrations which may result in iodine excess in humans.

References

Akers, R.M. 2008. Lactation and the Mammary Gland. Blackwell Publishing, Ames, Iowa.

Andersson, M., B. Takkouche, I. Engli, H. E. Allen, B. Benoist. 2005. Current global iodine status and progress over the last decade towards the elimination of iodine deficiency. Bulletin of the World Health Organization. 87:518-525

Archer, G.S., T.H. Friend, D. Caldwell, K. Ameiss, P.D. Krawczel, C. Iacono, H. Keen, T. Martin. 2008. Impacts of feeding several components of the seaweed *Ascophyllum nodosum* on transported lambs. Animal Feed Science and Technology. 140:258-271.

Bach. S.J, Y. Wang, T. A. McAllister. 2008. Effect of feeding sun-dried seaweed (*Ascophyllum nodosum)* on fecal shedding of *Esherichia coli* O157:H7 by feedlot cattle and on growth and performance of lambs. Animal Feed Science and Technology. 142:17-32

Bargo, F. L. D. Muller, J. E. Delahoy, T.W. Cassidy. 2002. Milk Response to Concetrate Supplementation of High Producing Dairy Cows Grazing at Two Pasture Allowances. J. Dairy Sci. 85:1777-1792

Berg, J. N., D. Padgitt, B. McCarthy. 1988. Iodine concentrations in milk of dairy cattle various amounts of iodine as ethylenediamine dihydroiodide. J. Dairy Sci. 71:3283-3291.

Berry, M. H., and K. L. Turk. 1944. The value of kelp meal in rations for dairy cattle. 861-866

Boruki Castro, S.I., R. Berthiaume, A. Robichaud, P. Lacasse. 2012. Effects of iodine intake and teat-dipping practices on milk iodine concentrations in dairy cows. J. Dairy Sci. 95:213-220

Bowman, B.A., R. M. Russell. 2006. Iodine. Present Knowledge in Nutrition. International Life Sciences Institute. Washington, D.C.

Collier. R. J. , G. E. Dahl, and M. J. VanBaale. 2006. Major Advances Associated with Environmental Effects on Dairy Cattle. J Dairy Sci. 89:1244-1253.

Cradduck. W. C. 2001. Infulence of *Ascophyllum nodosum* on Selenium and Antioxdants in Beef Cattle. A Thesis in Animal Science, Texas Tech University. Dairy Day Bulleti, Kansas State University.

Cvetkovic, B., M.J. Brouk, J. E. Shirley. 2004. Impact of dried seaweed meal on heat-stressed lactating dairy cattle.

Dahl, L., J. A. Opsahl, H. M. Meltzer, and K. Julshamn. 2003. Iodine concentration on Norwegian milk and dairy products. Br. J. Nutr. 90:679-685.

Erickson, P. S., S. P. Marston, M. Gemmel, J. Deming, R. G. Cabral, M. R. Murphy, and J. I. Marden. 2012. *Short Communication*: Kelp taste preferences by dairy calves. 95:856-858.

Ferreira, L.M.M., M. Olivan, M.A.M Rodrigues, K. Osoro, H. Dove, A. Dias-Da-Silva. 2004. Estimation of feed intake by cattle using controlled-release capsules containing n-alkanes or chromium sesquioxide. J. Agri. Sci. 142:225-234.

Fike, J.H., K. E. Saker, S. F. O'Keefe, N. G. Marriott, D. L. Ward, J. P. Fontenot, H. P. Veit. 2005. Effects of Tasco (a seaweed extract) and heat stress on N metabolism and meat fatty acids in wether lambs fed hays containing endophyte-infected fescue. Small Ruminant Research 60:237-245.

Fox, D.J., and T.P. Tylutiki. 1998. Accounting for the effects of the environment on nutrient requirements of dairy cattle. J. Dairy Sci. 81:3085-3095

Frame, J., J.F.L. Charlton, A.S. Laidlaw. 1998. White Clover. Page 72-73 in Temperate Forage Lgumes. CAB International, Wallingford, UK.

Franke, K., U. Meyer, H. Wagner, G. Flachowsky. 2009. Influence of various iodine supplementation levels and two iodine species on the iodine content of the milk of cows fed rapeseed meal or distillers dried grains with soluble as the protein source. J. Dairy Sci. 92:4514-4523.

Gardiner, G. E., A. J. Campbell, J. V. O'Doherty, E. Pierce, P. B. Lynch, F. C. Leonard,and C. Stanton. 2008. Effect of *Ascophllum nodosum* extract on growth performance, digestibility, carcass characteristics and selected intestinal microflora populations on grower-finsher pigs. Animal Feed Science and Technology. 141:259-273

Hamada, T. Estimating lower critical temperatures for dry and lactating dairy cows. Technical notes: J. Dairy Sci. 54:1704-1705.

Heo, Soo-Jin., Eun-Jun Park, Ki-Wan Lee, You-Jin Jeon. 2005. Antioxidant activities of enzymatic extracts from brown seaweeds. Bioresource Technology. 96:1613-1623.

Kannan, G. K. E. Saker, T.H. Terrill, B. Kouakou, S. Galipalli, S. Gelaye. 2007. Effect of seaweed extract supplementation in goats exposed to stimulated preslaughter stress. Small Ruminant Research. 73:221-227.

Karatzia, M., E. Christaki, E. Bonos, C. Karatzias, P. Florou-Paneri. 2012. The influence of dietary *Ascophyllum nodosum* on haematologic parameters of dairy cows. Italian Journal of Animal Science. 11:169-173.

Kellogg, D. W., K. Anschutz, and J. Pennington. 2006. Report of research trial with Tasco at Rose Ark Dairy in Arkansas during summer 2005. Arkansas Animal Science Department Report. 100-104

Kolver, E.S., L.D. Muller. 1998. Performance and nutrient intake of high producing Holstein cows consuming pasture or a total mixed ration. J. Dairy Sci. 81:1403-1411.

Macedo, R., R. M. Tarre, E. Ferreira, C. P. Rezende, J. M. Pereira, G. Cadisch, J. R. C. Rouws, B. J. R. Alves, S. Urquiaga, R. M. Boddey. 2010. Forage intake and botanical composition of feed for cattle fed *Brachiaria*/legume mixtures. Sci. Agric. (Piracicba, Braz.) 67:384-392.

Malossini, F., S. Bovolenta, E. Piasentier, C. Piras, and F. Marillotti.1995. Comparison of *n*-alkanes and chromium oxide methods for estimating herbage intake by grazing dairy cows. Animal Feed ScienceTechnology. 61: 155-165.

McBride, W. D., C. Greene. 2009. Characteristics, Costs and Issues for Organic Dairy Farming. ERR 82. USDA Economic Research Service, Washington, D.C.

Nudda. A., G. Battacone, M. Decandia, M. Acciaro, F. Aghini-Lombardi, M. Frigeri, and G. Pulina. 2009. The effect of dietary iodine supplementation in dairy goats on milk production traits and milk iodine content. J. Dairy Sci. 92:5133-5138

Pearce, E. N., S. Pino, X. He, H.R. Bazrafshan, S. L. Lee, L. E. Braverman. 2004. Sources of dietary iodine: bread, cow's milk and infant formula in the Boston area. Journal of Clinical Endocrinology and Metabolism. 89:3421-3424.

Pennington, J.A.T. 1990. Iodine concentration in U.S. milk variation due to time, season and region. J. Dairy Sci. 73:3421-3427.

Peyraud, J.L. 1997. **Techniques for measuring herbage intake of grazing ruminants: a review.** E. Spörndly, E. Burstedt, M. Murphy (Eds.) Managing High Yielding Dairy Cows at Pasture. Swedish University of Agricultural Sciences, Uppsala, Sweden. (3–23)

Pompeu, L.B., J.E. Williams, D.E. Spiers, R.L. Weaber, M.R. Ellersieck, K.M. Sargent, N.P. Feyerabend, H.L. Vellios, and F. Evans. 2011. Effect of *Ascophyllum nodosum* on alleviation of heat stress in dairy cows. The Professional Animal Scientist. 27:181-189

Roti, E. and Delgli Uberti, E. Iodine excess and hyperthyroidism. 2001. Thyroid. 11:493-500

Saladin, K.S. 2010. Anatomy and Physiology: The Unity of Form and Function. Fifth Edition. McGraw Hill. New York, NY

Seeley, R. H., and W. H Schlesinger. 2012. Sustainable seaweed cutting? The rockweed (*Ascophyllum nodosum)* industry of Maine and the Maritime Provinces. Ann. N.Y. Acad. Sci. ISSN 0077-8923

Sivakumar, A. V. N., G. Singh and V.P. Varshney. 2010. Antioxidants Supplementation on Acid Base Balance during Heat Stress in Goats. Asian-Aust. J. Anim. Sci. 11:1462-1468.

Smith, A. M., J.T. Reid. 1954. Use of chromic oxide as an indicator of fecal output for the purpose of determining the intake of pasture herbage by grazing cows. Department of Animal Husbandry, Cornell Universtiy, Ithaca, N.Y.

St-Pierre, N.R., B. Cobanov, G. Schnitkey. 2003. Economic losses from heat stress by U.S. livestock industries. J. Dairy Sci. 86:E52-E77.

Smit, H.J., H.Z. Taweel, B. M. Tas, S. Tamminga and A. Elgersma. 2005. Comparison of techniques for estimating herbage intake of grazing dairy cows. J. Dairy Sci. 88: 1827-1836

Swanson, E. W., J. K. Miller, F. J Mueller, C. S. Patton, J. A. Bacon, and N. Ramsey. 1990. Iodine in milk and meat of dairy cows fed different amounts of potassium iodide or ethylenediamine dihydroiodide. J. Dairy Sci. 73:398-405.

Tucker, C.B., A. R. Rogers, K. E. Schutz. 2008. Effect of solar radiation on dairy cattle behavior, use of shade and body temperature in a pasture-based system. Applied Animal Behaviour Sci. 109:141-154.

Valadares, R. F. D., G. A. Broderick, S. C. Valadares Filho and M. K. Clayton. 1999. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. J. Dairy Sci. 82:2686-2696.

Wang, Y., Z. Xu, S. J. Bach, T. A. McAllister. 2008. Effects of phlorotannins from *Ascophyllum nodosum* (brown seaweed) on *in-vitro* ruminal digestion of mixed forage or barley grain. Animal Feed Science and Technology. 145:375-395.

Wang, Y., Z. Xu, S. J. Bach, T. A. McAllister. 2009. Sensitivity of *Escherichia coli* to seaweed (*Ascophyllum nodosum)* phlorotannins and terrestrial tannins. Asian-Aust. J. Anim. Sci. 22:238-245

Yamaguchi, S., Ninomiya, K. 2000. The use and utility of glutamates as flavoring agents in food. J. of Nutrition. 921S-926S

Zimmerman, M.B., Y. Ito, S. Y. Hess, K. Fujieda and L. Molinari. 2005. High thyroid volume in children with excess dietary iodine intakes. Am. J. Clin. Nutr. 81:840-844.

TABLES AND FIGURES

Table 1. Nutritional composition of *Ascophyllum nodosum* meal (ANOD), TMR, concentrate blend, and baleages fed to early lactation dairy cows

				Baleages	
Item	ANOD	TMR[1]	Concentrate[2]	Mixed-mostly grass	Mixed-mostly legume
DM, % of fresh matter	93.23	60.75	89.53	46.64	51.54
CP, % of DM	10.20	16.28	17.63	14.95	19.68
ADIN, % of DM	0.85	0.24	NA[3]	0.16	0.23
NDIN, % of DM	0.88	NA	NA	NA	NA
Soluble protein, % of CP	57.00	49.75	NA	NA	NA
NDF, % of DM	53.90	48.70	13.93	62.10	53.73
ADF, % of DM	39.90	34.40	7.00	39.20	38.35
Lignin, % of DM	20.00	NA	NA	NA	NA
Ash, % of DM	25.90	7.97	9.93	8.34	9.94
AIA[4], % of DM	0.21	0.71	0.42	0.73	0.86
Crude fat, % of DM	2.30	3.78	3.70	3.95	4.00
ESC[5], % of DM	3.30	NA	NA	NA	NA
Starch, % of DM	0.70	NA	NA	NA	NA
NFC[6], % of DM	13.10	23.27	54.81	10.66	12.65
$NE_{L,}$ Mcal/kg	0.55	1.48	1.76	1.12	1.24

[1]The TMR contained (DM basis): 31.8% mixed-mostly grass baleage, 32.4% mixed-mostly legume baleage, and 35.8% concentrate blend.
[2]Ingredients found in concentrate blend in Table 5
[3]NA = not analyzed.
[4]AIA = acid insoluble ash.
[5]ESC = ethanol soluble carbohydrates.
[6]Calculated as 100 – [(%CP – %NDICP) + %NDF + %crude fat + %ash] for ANOD and as 100 – (%CP + %NDF + %crude fat + %ash) for TMR, concentrate, and baleages.

Table 2. Mineral and vitamin composition of *Ascophyllum nodosum* meal (KM), TMR, concentrate blend, and baleages fed to early lactation dairy cows

				Baleages	
Item	KM	TMR[1]	Concentrate[2]	Mixed-mostly grass	Mixed-mostly legume
Ca, % of DM	1.31	0.61	0.95	0.45	0.75
P, % of DM	0.25	0.45	0.60	0.37	0.46
Mg, % of DM	0.69	0.33	0.78	0.21	0.22
K, % of DM	3.53	2.39	1.14	2.94	3.32
Na, % of DM	3.90	0.47	1.26	0.10	0.08
Fe, mg/kg of DM	287	277.3	526.0	201	205
Zn, mg/kg of DM	9.00	81.75	254.0	24.5	35.5
Cu, mg/kg of DM	3.00	15.25	40.00	8.75	8.75
Mn, mg/kg of DM	20.00	67.5	183.0	39.00	30.50
Mo, mg/kg of DM	<0.10	2.25	1.53	3.08	3.60
S, % of DM	2.84	0.25	0.34	0.22	0.27
Cl, % of DM	4.70	0.68	1.24	0.48	0.28
DCAD, mEq/100 g of DM	-49.00	0.47	28.00	52.00	63.00
I, mg/kg of DM	820	3.14	0.20	0.31	0.20
Cr, mg/kg of DM	1.04	NA[3]	NA	NA	NA
Se, mg/kg of DM	<0.41	NA	NA	NA	NA
Co, mg/kg of DM	1.55	NA	NA	NA	NA
As, mg/kg of DM	28.30	NA	NA	NA	NA
Thiamin, µg/kg of DM	0.68	NA	NA	NA	NA
Vitamin B_6[4], µg/kg of DM	0.69	NA	NA	NA	NA
Niacin[5], µg/kg of DM	29.58	NA	NA	NA	NA
Riboflavin, µg/kg of DM	4.92	NA	NA	NA	NA
Folates, µg/kg of DM	990.7	NA	NA	NA	NA
Vitamin B_{12}, µg/kg of DM	23.45	NA	NA	NA	NA

[1]The TMR contained (DM basis): 31.8% mixed-mostly grass baleage, 32.4% mixed-mostly legume baleage, and 35.8% concentrate blend.

[2]Ingredients found in concentrate blend shown in Table 5

[3]NA = not analyzed.

[4]Vitamin B6 = pyridoxamine + pyridoxal + pyridoxine.

[5]Niacin = nicotinic acid + nicotinamide.

Table 3. Ingredients in concentrate blend.

Ingredient	% as fed basis
Organic Corn Meal	46.7
Organic barley	22.0
Organic soybean meal	11.3
Organic wheat middlings	5.2
Organic roasted soybean	8.0
Salt	1.50
Sodium bicarbonate	1.25
Limestone	1.10
DIKAL 21[1]	0.82
Magnesium oxide	0.72
Potassium sulfate	0.44
Selenium	0.41
Mineral premix[2]	0.34
A-D-E vitamin premix[3]	0.29

[1] DIKAL 21 contained (19% CA and 21 %P)

[2] The mineral premix provided (guaranteed analysis): 30% of Ca, 790 mg/kg of I, 675 mg/kg of Co, 6.0 g/kg of Cu, 4.0% of Zn, and 2.5% of Mn

[3]The A-D-E vitamin premixes provided (guaranteed analysis): 6,062,721 IU/kg of vitamin A, 1,653,450 IU/kg of vitamin D, 25,353 IU/kg of vitamin E

Table 4. Fatty acids composition of *Ascophyllum nodosum* meal (KM) and TMR, fed to early lactation dairy cows

	KM	TMR
Fatty acid	--------g/100 g of total FA--------	
14:0	11.77	0.42
16:0	14.75	17.55
16:1	1.44	0.20
17:0	0.00	0.20
18:0	0.61	2.67
cis-9 18:1	30.22	11.37
cis-11 18:1	0.27	0.72
cis-9, *cis*-12 18:2	7.98	31.00
γ-*cis*-9, *cis*-12, *cis*-15 18:3	0.29	0.016
α-*cis*-9, *cis*-12, *cis*-15 18:3	4.01	22.23
20:0	0.040	0.80
cis-13 20:1	0.00	0.26
cis-11, *cis*-14 20:2	1.64	0.084
20:3	0.77	0.00
C22:0	1.69	0.92
cis-7, *cis*-10, *cis*-13, *cis*-16 22:4	0.00	0.11
24:0	5.15	0.48
cis-15 24:1	0.00	0.51
Unidentified	19.38	10.48

Table 5. Amino acids composition of *Ascophyllum nodosum* meal fed to early lactation dairy cows

Amino Acid	---% of CP---
Arginine	2.63
Histidine	1.09
Isoleucine	2.35
Leucine	3.80
Lysine	3.17
Methionine	1.18
Phenylalanine	2.54
Threonine	2.80
Tryptophan	0.45
Valine	3.17
Alanine	4.08
Asparagine	7.33
Cystine	1.63
Glutamic Acid	16.02
Glycine	3.08
Ornithine	0.36
Proline	1.90
Serine	2.35
Taurine	0.54
Tyrosine	1.18

Table 6. Least square means for DMI, milk yield, concentrations and yields of milk components, feed efficiency, and body weight change in organic Jersey cows fed incremental dietary levels of kelp meal

	Dietary Levels of Kelp Meal (as fed)					Contrasts (*P*-value)[1]	
Item	0 g	57 g	113 g	170 g	SEM	Linear	Quadratic
DMI, kg/d	17.52	18.07	18.06	17.62	0.60	0.82	0.09
Milk yield, kg/d	16.27	16.23	16.43	15.77	0.70	0.27	0.24
Milk fat, %	5.08	5.10	5.24	5.09	0.16	0.74	0.28
Milk fat, kg/d	0.84	0.85	0.88	0.86	0.04	0.35	0.55
Milk protein, %	3.68	3.70	3.64	3.63	0.07	0.27	0.79
Milk protein, kg/d	0.61	0.62	0.61	0.62	0.03	0.86	0.95
Milk lactose, %	4.69	4.71	4.68	4.70	0.03	0.96	0.97
Milk lactose, kg/d	0.78	0.79	0.79	0.80	0.04	0.53	0.96
Milk SNF, %	9.18	9.16	9.18	9.15	0.05	0.42	0.99
Milk SNF, kg/d	1.53	1.53	1.54	1.55	0.07	0.55	0.94
Milk TS, %	14.27	14.26	14.42	14.25	0.18	0.87	0.36
Milk TS, kg/d	2.40	2.38	2.35	2.42	0.12	0.91	0.59
4% FCM[2], kg/d	18.12	17.87	18.71	18.27	0.97	0.63	0.88
ECM[3], kg/d	20.90	20.83	21.31	20.95	1.02	0.80	0.80
MUN, mg/dL	12.10	11.44	11.58	11.60	0.46	0.18	0.12
Milk yield/DMI, kg/kg	0.94	0.90	0.91	0.90	0.03	0.25	0.54
4% FCM/DMI, kg/kg	1.04	0.99	1.03	1.04	0.04	0.72	0.45
ECM/DMI, kg/kg	1.28	1.28	1.30	1.32	0.04	0.28	0.62
Milk N/N intake, g/g	19.57	19.14	18.93	19.53	0.68	0.89	0.38
BW change, kg/d	0.22	0.03	0.54	-2.21	1.97	0.45	0.53
Final BW, kg	442.1	440.3	442.4	440.3	8.07	0.56	0.92

[1]Probability of linear and quadratic effects for incremental dietary levels of kelp meal; significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

[2]4% FCM = [0.40 × milk yield (kg/d)] + [15 × milk fat yield (kg/d)] (Gaines and Davidson, 1923).

[3]ECM = [0.327 × milk yield (kg/d)] + [12.95 × fat yield (kg/d)] + [7.2 × protein yield (kg/d)] (Orth, 1992)

Table 7: Least square means for plasma and serum parameters analyzed in organic Jersey cows fed incremental dietary levels of kelp meal

	Dietary Levels of Kelp Meal (as fed)					Contrasts (*P*-value)[1]	
Item	0 g	57 g	113 g	170 g	SEM	Linear	Quadratic
PUN, mg/dL	17.41	17.98	17.02	17.19	0.59	0.43	0.66
Plasma NEFA, mEq/L	163.8	144.1	135.0	131.9	11.86	0.05	0.48
Serum cortisol, ng/mL	74.76	70.13	59.58	61.55	8.52	0.08	0.60
Serum T_3, ng/mL	1.10	1.05	1.11	1.09	0.05	0.84	0.70
Serum T_4, ng/mL	48.60	49.28	49.56	46.14	1.53	0.25	0.14

[1]Probability of linear and quadratic effects for incremental dietary levels of kelp meal; significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

Table 8. Least square means for iodine intake, concentration and yield of milk iodine, apparent recovery of milk iodine in organic Jersey cows fed incremental dietary levels of kelp meal

	Dietary Levels of Kelp Meal (as fed)					Contrasts (*P*-value)[1]	
Item	0 g	57 g	113 g	170 g	SEM	Linear	Quadratic
Kelp meal iodine intake[2], mg/d	-	42.71	86.17	129.6	0.00	<0.001	1.00
TMR iodine intake[3], mg/d	54.85	56.46	57.53	55.81	2.07	0.48	0.19
Total dietary iodine intake, mg/d	54.85	99.16	143.7	185.5	2.07	<0.001	0.31
Milk iodine, µg/L	177.6	602.3	1,014.8	1,369.9	69.91	<0.001	0.48
Milk iodine, mg/d	2.82	9.33	16.11	20.58	0.86	<0.001	0.12
Apparent recovery of milk iodine[4], %	-	21.85	18.69	15.88	1.21	<0.001	0.85

[1]Probability of linear and quadratic effects for incremental dietary levels of kelp meal; significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

[2]The treatment with 0 g of kelp meal was not included in the statistical model because no iodine from kelp meal was supplied.

[3]Calculated based on TMR iodine intake plus iodine provided by the daily dose of concentrate mash (450 g as fed), which was fed after the a.m. milking with or without kelp meal.

[4]Apparent recovery of milk iodine = milk iodine (mg/d) ÷ iodine intake from kelp meal (mg/d) × 100.

Table 9. Least square means for intakes and apparent total tract digestibilities of DM, OM, NDF, ADF, and N in organic Jersey cows fed incremental dietary levels of kelp meal

	Dietary Levels of Kelp Meal (as fed)					Contrasts (*P*-value)[1]	
Item	0 g	57 g	113 g	170 g	SEM	Linear	Quadratic
	----------Intake, kg/d----------						
DM	17.52	18.07	18.06	17.62	0.60	0.82	0.09
OM	16.15	16.68	16.61	16.33	0.56	0.70	0.14
NDF	8.57	8.61	8.73	8.45	0.21	0.66	0.21
ADF	6.05	6.09	6.18	5.98	0.20	0.70	0.15
N	473.9	476.4	480.4	465.1	15.33	0.45	0.19
	----------Digestibility, % of intake----------						
DM	70.63	70.53	71.48	68.97	0.93	0.27	0.14
OM	72.30	72.86	73.63	71.44	0.86	0.60	0.08
NDF	68.71	69.28	70.16	67.35	1.09	0.48	0.10
ADF	68.58	69.27	70.49	65.94	1.29	0.23	0.04
N	70.02	69.50	70.27	67.45	1.05	0.09	0.20

Table 10. Least square means for saturated and unsaturated fatty acids in cows fed incremental dietary levels of kelp meal

	Dietary Levels of Kelp Meal (as fed)					Contrasts (*P*-value)[1]	
FA	0 g	57 g	113 g	170 g	SEM	Linear	Quadratic
	----------FA, % of total milk FA----------						
C4:0	3.27	3.32	3.35	3.30	0.10	0.44	0.18
C5:0	0.019	0.021	0.020	0.022	0.002	0.39	0.98
C6:0	2.38	2.39	2.41	2.40	0.06	0.55	0.65
C7:0	0.026	0.027	0.027	0.031	0.002	0.05	0.44
C8:0	1.46	1.45	1.47	1.47	0.03	0.66	0.92
C9:0	0.033	0.033	0.031	0.032	0.002	0.23	0.99
C10:0	3.46	3.41	3.39	3.45	0.10	0.83	0.36
11-Cyclohexyl C11:0	0.079	0.080	0.073	0.078	0.006	0.53	0.51
C11:0	0.38	0.37	0.38	0.38	0.02	0.48	0.53
C12:0	4.02	3.95	3.91	3.98	0.14	0.65	0.36
C13:0	0.20	0.19	0.19	0.20	0.01	0.65	0.10
iso C13:0	0.023	0.025	0.022	0.024	0.002	0.80	0.80
anteiso C13:0	0.099	0.096	0.10	0.094	0.006	0.36	0.64
C14:0	12.51	12.39	12.27	12.49	0.29	0.70	0.12
iso C14:0	0.16	0.15	0.15	0.16	0.01	0.46	0.05
C15:0	1.20	1.17	1.13	1.18	0.03	0.12	0.03
iso C15:0	0.25	0.25	0.24	0.25	0.01	0.53	0.82
anteiso C15:0	0.40	0.40	0.38	0.40	0.02	0.88	0.35
C16:0	35.33	35.35	35.20	35.19	0.95	0.78	0.97
iso C16:0	0.34	0.34	0.36	0.33	0.02	0.57	0.11
C17:0	0.66	0.65	0.65	0.65	0.01	0.26	0.44
iso C17:0	0.18	0.19	0.18	0.18	0.01	0.97	0.77
anteiso C17:0	0.32	0.32	0.32	0.32	0.01	0.51	0.73
C18:0	10.09	10.09	9.84	9.93	0.75	0.46	0.84
iso C18:0	0.016	0.009	0.014	0.011	0.002	0.39	0.12

Table 10 continued. Least square means for saturated and unsaturated fatty acids in cows fed incremental dietary levels of kelp meal

	Dietary Levels of Kelp Meal (as fed)					Contrasts (*P*-value)[1]	
FA	0 g	57 g	113 g	170 g	SEM	Linear	Quadratic
C20:0	0.16	0.16	0.15	0.16	0.01	0.75	0.22
C21:0	0.022	0.022	0.022	0.021	0.00	0.71	0.84
C22:0	0.058	0.059	0.055	0.061	0.00	0.75	0.55
ΣSFA	77.15	76.91	76.30	76.81	0.63	0.34	0.33
cis-9 C14:1	0.96	0.94	0.97	0.97	0.07	0.59	0.73
trans-9 C14:1	0.008	0.009	0.010	0.011	0.001	0.06	0.30
cis-9 C16:1	1.24	1.21	1.33	1.23	0.10	0.66	0.41
ΣC16:1 FA[2]	1.51	1.43	1.62	1.50	0.11	0.55	0.33
cis-9 C17:1	0.13	0.13	0.14	0.13	0.01	0.99	0.30
cis-9 C18:1	13.15	13.35	13.74	13.37	0.47	0.41	0.32
Σ*cis* C16:1 to *cis* C18:1 FA[3]	15.28	15.45	16.03	15.48	0.50	0.36	0.22
trans-10 C18:1	0.26	0.26	0.26	0.29	0.02	0.31	0.58
trans-11 C18:1	1.12	1.13	1.07	1.09	0.07	0.45	0.90
trans-11/trans-10 C18:1	4.60	4.33	4.18	3.95	0.48	0.26	0.96
Σ*trans* C18:1 FA[4]	1.87	1.87	1.81	1.85	0.08	0.62	0.75
ΣC18:1 FA[5]	16.23	16.42	16.78	16.43	0.54	0.52	0.41
cis-9, *cis*-11 CLA	0.030	0.029	0.026	0.028	0.003	0.45	0.63
trans-7, *trans*-9/*trans*-10, *trans*-12 CLA	0.014	0.011	0.013	0.013	0.002	0.90	0.38
trans-9, *cis*-11 CLA	0.002	0.003	0.002	0.003	0.001	0.63	0.68
cis-9, *trans*-11 CLA	0.48	0.47	0.46	0.48	0.028	0.92	0.48
trans-11, *trans*-13 CLA	0.008	0.011	0.007	0.009	0.002	0.92	0.61
ΣCLA	0.53	0.52	0.51	0.53	0.03	0.86	0.48
ΣC18:2 FA[6]	2.55	2.53	2.63	2.58	0.09	0.28	0.70
cis-9, *cis*-12, *cis*-15 C18:3	0.46	0.47	0.48	0.47	0.02	0.21	0.32
ΣC18:3 FA[7]	0.49	0.49	0.51	0.50	0.02	0.21	0.57
cis-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17 C20:5	0.073	0.074	0.076	0.074	0.00	0.28	0.97

Table 10 continued. Least square means for SFA and unsaturated FA in cows fed incremental dietary levels of kelp meal

	Dietary Levels of Kelp Meal (as fed)					Contrasts (*P*-value)[1]	
FA	0 g	57 g	113 g	170 g	SEM	Linear	Quadratic
ΣC20:1 to C22:6 FA[8]	0.39	0.40	0.42	0.38	0.02	0.65	0.01
Σn-6 FA[9]	1.82	1.82	1.93	1.85	0.07	0.20	0.29
Σn-3 FA[10]	0.58	0.58	0.61	0.59	0.02	0.22	0.25
n-6/n-3	3.17	3.13	3.16	3.14	0.08	0.85	0.88
ΣMUFA	18.98	19.14	19.68	19.19	0.55	0.43	0.33
ΣPUFA	3.30	3.29	3.42	3.35	0.11	0.28	0.52
Σunsaturated FA	22.28	22.43	23.10	22.53	0.62	0.38	0.33

[1]Probability of linear and quadratic effects for incremental dietary levels of kelp meal; significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

[2]*cis*-7 C16:1 + *cis*-8 C16:1 + *cis*-9 C16:1 + *trans*-9 C16:1 + *cis*-10/*trans*-13 C16:1 + *cis*-11 C16:1 + C16:1 isomer.

[3]Σ*cis* C16:1 (see list above) + *cis*-8 C17:1 + *cis*-9 C17:1 + *cis*-9 C18:1 + *cis*-11 C18:1 + *cis*-12 C18:1 + *cis*-13 C18:1 + *cis*-15 C18:1 + *cis*-16 C18:1.

[4]*trans*-4 C18:1 + *trans*-5 C18:1 + *trans*-6-8 C18:1 + *trans*-9 C18:1 + *trans*-10 C18:1 + *trans*-11 C18:1 + *trans*-12 C18:1.

[5]Σ*trans* C18:1 FA (see list above) + Σcis C18:1 FA (see list above) +*cis*-6-8/13/*trans*-14 C18:1 + *cis*-14/*trans*-16 C18:1.

[6]ΣCLA + *cis*-9, *cis*-12 C18:2 + *cis*-9, *trans*-13/*trans*-8, *cis*-12 C18:2 + *cis*-9, *trans*-14 C18:2 + *cis*-12, *trans*-16 C18:2 + *trans*-9, *cis*-12 C18:2 + *trans*-11, *cis*-15 C18:2 + *trans*-12, *cis*-15 C18:2 + *trans*-9, *trans*-12 C18:2 + *trans*-12, *trans*-14 C18:2.

[7]*cis*-6, *cis*-9, *cis*-12 C18:3 + *cis*-9, *cis*-12, *cis*-15 C18:3 + *trans*-9, *trans*-12, *cis*-15 C18:3.

[8]*cis*-9 C20:1 + *cis*-11 C20:1 + *cis*-11, *cis*-14 C20:2 + *cis*-5, *cis*-8, *cis*-11 C20:3 + *cis*-11, *cis*-14, *cis*-17 C20:3 + *cis*-5, *cis*-8, *cis*-11, *cis*-14 C20:4 + *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17 C20:5 + *cis*-13 C22:1 + *trans*-13 C22:1 + *cis*-7, *cis*-10, *cis*-13, *cis*-16 C22:4 + *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19 C22:5 + *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19 C22:6.

[9]*cis*-9, *cis*-12 C18:2 + *cis*-6, *cis*-9, *cis*-12 C18:3 + *cis*-11, *cis*-14 C20:2 + *cis*-5, *cis*-8, *cis*-11 C20:3 + *cis*-5, *cis*-8, *cis*-11, *cis*-14 C20:4 + *cis*-7, *cis*-10, *cis*-13, *cis*-16 C22:4.

[10]*cis*-9, *cis*-12, *cis*-15 C18:3 + *cis*-11, *cis*-14, *cis*-17 C20:3 + *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17 C20:5 + *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19 C22:5 + *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19 C22:6.

Table 11. Urinary concentration and excretion of purine derivatives (PD = allantoin plus uric acid), urinary excretion of N and urea N, and estimated bacterial protein synthesis in organic Jersey cows fed incremental dietary levels of kelp meal

	Dietary Levels of Kelp Meal (as fed)					Contrasts (*P*-value)[1]	
Item	0 g	57 g	113 g	170 g	SEM	Linear	Quadratic
Creatinine, m*M*	3.41	3.21	3.32	3.47	0.16	0.59	0.16
Allantoin, m*M*	5.76	6.60	6.05	5.94	0.34	0.96	0.12
Uric acid, m*M*	0.35	0.30	0.37	0.38	0.05	0.31	0.50
PD, m*M*	6.11	6.90	6.43	6.32	0.34	0.85	0.15
PD/creatinine	1.85	2.20	2.00	1.86	0.14	0.70	0.03
Allantoin, m*M*/d	221.8	271.7	241.2	221.7	18.30	0.71	0.01
Uric acid, m*M*/d	13.94	12.47	15.33	15.18	2.58	0.42	0.73
PD[2], m*M*/d	235.7	284.1	256.4	236.9	19.35	0.70	0.02
N excretion, g/d	148.5	184.2	159.8	159.6	8.83	0.76	0.03
N excretion, % of N intake	31.89	39.53	34.04	35.86	1.95	0.37	0.10
Urea N excretion, g/d	129.3	143.1	141.4	132.3	8.65	0.93	0.16
Urea N excretion, % urinary N	86.11	77.80	84.97	83.52	2.62	0.94	0.16
Urea N excretion, % of N intake	27.33	30.74	30.12	29.82	2.09	0.56	0.39
Bacterial N synthesis[3], g/d	171.7	211.3	198.9	172.8	-	-	-

[1]Probability of linear and quadratic effects for incremental dietary levels of kelp meal; significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

[2]Calculated based on the PD to creatinine ratio (Chizzotti et al., 2008) assuming a constant creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999).

[3]Bacterial N synthesis, g/d = (70 × purines absorption) ÷ (0.83 × 0.116 × 1,000), where 70 represents the concentration of N in purines (mg N/mmol), 0.83 is the intestinal digestibility of bacterial purines, and 0.116 is the purine N/total bacterial N ratio (Chen and Gomes, 1992); purines absorption = 0.85 × purines absorption + 0.512 × $BW^{0.75}$, where 0.85 is the efficiency of intestinal absorption of purines (Verbic et al., 1980), and 0.512 × $BW^{0.75}$ is the endogenous contribution of purines (Gonzalez-Ronquillo et al., 2003)

Table 12: Nutrient composition of baleage, concentrate, KM and TMR fed during pasture supplementation of KM trial

	Units	Baleage	Concentrate	Kelp	TMR	Cr Pellet
Crude Protein	% of DM	11.975	12.15	10.3	11.75	17.7
ADICP	% of DM	1.35	0.4	5.1	1.075	1.1
soluble protein	% of CP	49.25	27.5	54	36.5	35
NDICP	% of DM	2.45	1.425	5.6	2.225	2.1
ADF	% of DM	41.95	3.7	20.8	27.55	10
NDF	% of DM	61.825	10.1	39.2	43.575	17
Lignin	% of DM	6.375	1.525	12.2	4.95	3.2
NFC	% of DM	19.55	68.15	27.7	36.725	55.7
Starch	% of DM	1.25	57.125	0.4	21.275	41.2
ESC simple sugars	% of DM	6.6	3.15	3.9	7.9	4.3
Crude Fat	% of DM	2.85	2.225	2.4	2.675	3
Ash	% of DM	6.235	8.825	26.13	7.4975	8.72
Calcium	% of DM	84.75	1.0225	1.28	0.6025	1.07
Phosphorus	% of DM	0.4125	0.5925	0.21	0.35	0.43
Magnesium	% of DM	0.25	0.7	0.8	0.395	0.38
Potassium	% of DM	0.2025	0.5375	2.57	1.5375	0.80
Sodium	% of DM	1.9425	1.22275	3.591	0.5717	0.74
Iron	mg/kg of DM	0.104	425.25	403	248.75	292
Zinc	mg/kg of DM	131.5	160.5	11	81.75	141
Copper	mg/kg of DM	25	24.25	4	14	22
Manganese	mg/kg of DM	8.75	108.5	24	71.5	96
Molybdenum	mg/kg of DM	40.5	1.05	0.9	1.525	1.1
Sulfur	% of DM	2.075	0.245	2.71	0.2175	0.20
Chloride	% of DM	0.17	1.195	4.73	0.7625	0.87
Iodine	mg/kg of DM	<4	<4	727	<4	<4

Table 13. Ingredient Composition of Concentrate Blend

Ingredient	Concentrate Blend	Chromium Pellet
Organic corn meal	51.97	37.98
Organic barley	20.68	0.00
Organic wheat	10.00	5.68
Organic peas	5.77	10.00
Organic soy meal	4.00	4.72
Organic midds	0.00	18.50
Organic linseed meal	0.00	9.00
Organic alfalfa meal	0.00	4.00
Organic molassess	0.00	3.00
Salt	1.55	1.13
Sodium bicarbonate	1.50	1.00
Limestone	1.33	2.13
DIKAL 21[1]	1.12	0.00
Magnesium oxide	0.98	0.45
Potassium sulfate	0.32	0.06
Selenium	0.17	0.10
Mineral premix[2]	0.33	0.22
A-D-E vitamin premix[3]	0.28	0.29
Sodium bentonite	0.00	0.50
Redmond conditioner	0.00	0.50
Chromium oxide	0.00	0.74

[1] DIKAL 21 contained (19% CA and 21 %P)

[2] The mineral premix provided (guaranteed analysis): 30% of Ca, 790 mg/kg of I, 675 mg/kg of Co, 6.0 g/kg of Cu, 4.0% of Zn, and 2.5% of Mn

[3]The A-D-E vitamin premixes provided (guaranteed analysis): 6,062,721 IU/kg of vitamin A, 1,653,450 IU/kg of vitamin D, 25,353 IU/kg of vitamin E

Table 14: Nutrient composition of pasture during each of the four sampling periods

	units	Period 1 July	Period 2 August	Period 3 September	Period 4 October	Average July-October
Crude Protein	%	13.9	16.5	17.5	18.1	16.5
Available	%	12.7	15.2	15.9	16.2	15
ADICP	%	1.2	1.3	1.5	1.9	1.475
Adjusted Crude	%	13.9	16.5	16.9	17.2	16.125
soluble protein	%	27	28	31	23	27.25
NDICP	%	6.5	7.4	8.2	9.8	7.975
ADF	%	33	35.1	34.1	33.3	33.875
NDF	%	59	59.6	66	63.7	62.075
Lignin		5.5	5.4	4.6	8.6	6.025
NFC		21.2	18.9	12.3	15.2	16.9
Starch		1.7	0.3	0.4	0.7	0.775
ESC simple		9.1	6.4	7.6	5.7	7.2
Crude Fat	%	3.6	3.9	3.3	3.8	3.65
Ash		8.81	8.4	9.08	9.11	8.85
TDN	%	60	61	59	54	58.5
NEL	Mcal/lb	0.55	0.56	0.49	0.47	0.5175
NEM	Mcal/lb	0.56	0.57	0.53	0.47	0.5325
NEG	Mcal/lb	0.3	0.31	0.27	0.22	0.275
Relative Feed		100	96	88	92	94
Calcium	%	0.61	0.64	0.58	0.57	0.6
Phosphorus	%	0.41	0.36	0.32	0.33	0.355
Magnesium	%	0.29	0.32	0.3	0.29	0.3
Potassium	%	2.53	2.11	2.36	2.29	2.3225
Sodium	%	0.042	0.112	0.064	0.04	0.0645
Iron	ppm	309	292	360	598	389.75
Zinc	ppm	26	30	29	37	30.5
Copper	ppm	8	10	9	10	9.25
Manganese	ppm	33	63	44	62	50.5
Molybdenum	ppm	4.5	1.4	2.1	2.3	2.575
Sulfur	%	.21	.28	.24	.25	.245
Chloride	%	.23	.53	.58	.81	.5375
DCAD	mEq/100g	47	27	32	22	32

Table 15. Pasture management during each of the four sampling periods

	Period 1	Period 2	Period 3	Period 4	Average
Pasture Management	July	August	September	October	July-October
Pasture allowance, kg DM/cow/d	21.09	7.84	11.28	11.12	12.83
Area, m^2/cow/d	94.72	46.15	55.89	91.54	72.07
Pregrazing herbage mass, kg DM/ha	2227	1699	1580	1215	1680
Postgrazing herbage mass, kg DM/ha	1267	715	1209	768	990
Grass, % of DM	63.77	51.18	66.9	47.25	57.28
Legume, % of DM	24.84	17.69	9.64	13.85	16.51
Weed, % of DM	9.26	15.44	9.6	11.93	11.56
Dead, % of DM	1.08	11.15	13.86	26.97	13.27

Table 16: Milk production and components, blood analysis and heat stress parameters of lactating organic Jersey cows fed 0 g (control = C) or 56.8 g of kelp meal (KM) during the grazing season

	Experiment			Period 1		Period 2		Period 3		Period 4			*P*-value[1]	
	C	KM	SEM	C	KM	C	KM	C	KM	C	KM	SEM	Trt	T x P
Milk yield, kg/d	12.44	12.98	0.57	14.39	15.20	13.05	12.34	11.62	13.05	10.70	11.34	0.54	0.52	0.20
4% FCM[2], kg/d	13.06	13.72	0.62	15.31	16.32	13.03	12.39	12.03	13.88	11.84	12.27	0.61	0.43	0.16
ECM[3] kg/d	14.19	1487	0.68	16.26	17.40	14.10	13.26	13.11	15.22	13.11	13.61	0.66	0.49	0.14
Milk fat, %	4.36	4.51	0.22	4.41	4.50	3.96	4.15	4.21	4.48	4.85	4.91	0.19	0.64	0.88
Milk fat, kg/d	0.54	0.57	0.03	0.64	0.68	0.52	0.50	0.49	0.58	0.50	0.52	0.03	0.46	0.43
Milk protein, %	3.56	3.54	0.08	3.21	3.20	326	3.26	3.74	3.75	4.04	3.97	0.07	0.78	0.87
Milk protein, kg/d	0.44	0.45	0.02	0.46	0.50	0.43	0.39	0.43	0.48	0.43	0.45	0.03	0.58	0.02
Milk lactose, %	4.69	4.70	0.02	4.71	4.74	4.71	4.70	4.57	4.62	4.77	4.75	0.02	0.70	0.36
Milk lactose, kg/d	0.57	0.60	0.03	0.68	0.72	0.62	0.57	0.53	0.60	0.47	0.49	0.04	0.59	0.02
Milk SNF, %	9.12	9.24	0.13	8.84	8.87	8.1	8.81	9.27	9.31	9.56	9.99	0.14	0.44	0.51
Milk SNF, kg/d	1.12	1.16	0.05	1.27	1.34	1.15	1.06	1.08	1.21	0.99	1.01	0.07	0.67	0.14
Milk total solids, %	13.48	13.58	0.31	13.24	13.16	12.77	12.96	13.48	13.79	14.41	14.40	0.25	0.97	070
Milk total solids, kg/d	1.66	1.70	0.08	1.91	1.98	1.67	1.56	1.57	1.79	1.48	1.47	0.08	0.71	0.15
MUN, mg/dL	11.61	12.11	0.43	12.26	14.41	12.23	12.00	13.15	13.00	8.79	9.01	0.44	0.40	0.08
Milk iodine	138.2	592.1	45.95	140.3	415.5	110.8	566.3	101.7	446.7	199.8	939.8	40.11	<0.001	<0.001
Plasma T_3, ng/mL	0.89	0.81	0.04	0.95	0.99	0.77	0.73	0.91	0.78	0.92	0.75	0.04	0.19	<0.001
Plasma T_4, ng/mL	41.92	39.69	0.98	36.05	38.97	40.19	38.11	46.56	41.94	44.88	39.74	1.45	0.14	0.04
Plasma cortisol, ng/mL	95.66	111.1	11.64	90.59	113.2	78.90	113.2	82.70	93.75	130.5	144.0	10.34	0.33	0.96
Plasma glucose	60.9	59.86	1.54	64.99	66.45	60.21	56.13	60.41	59.62	57.97	57.24	1.42	0.59	0.36
Plasma NEFA, µg/dL	121.2	110.8	5.49	119.2	89.82	135.8	128.4	119.8	108.3	109.9	116.9	12.04	0.22	0.79
a.m. Resp. rate, breath/mim	34.9	33.8	0.73	34.2	31.6	38.1	36.7	36.8	38.1	30.4	28.7	1.27	0.31	0.40
p.m. Resp. rate, breath/mim	54.0	54.8	1.59	70.7	70.5	61.3	63.0	51.3	52.9	32.8	32.7	2.45	0.75	0.94
a.m. Rectal temperature, °C	37.8	39.9	0.05	37.6	37.7	37.7	37.8	37.9	38.0	38.2	38.3	0.07	0.23	0.71
p.m. Rectal temperature, °C	38.6	38.7	0.05	38.8	38.7	38.6	38.6	38.6	38.8	38.5	38.6	0.07	0.31	0.48

[1] Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$

[2]4% FCM = [0.40 × milk yield (kg/d)] + [15 × milk fat yield (kg/d)] (Gaines and Davidson, 1923)

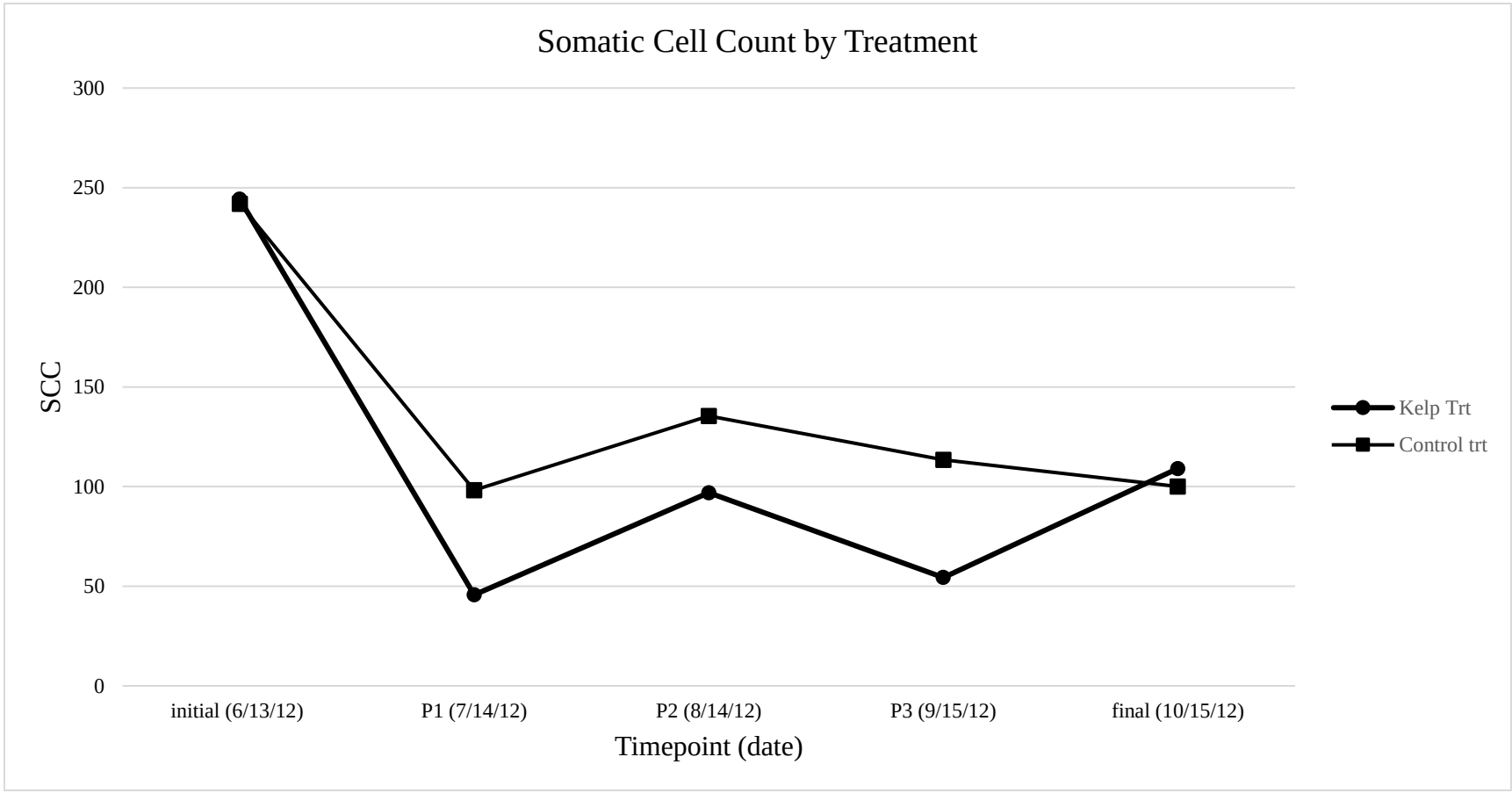
[3]ECM = [0.327 × milk yield (kg/d)] + [12.95 × fat yield (kg/d)] + [7.2 × protein yield (kg/d)] (Orth, 1992

Table 17: Dry matter intake, nutrient intake, nutrient digestibility and feed efficiency of lactating organic Jersey cows fed 0 g (control = C) or 56.8 g of kelp meal (KM) during the grazing season

	Experiment			Period 1		Period 2		Period 3		Period 4			*P*-value[1]	
	C	KM	SEM	C	KM	C	KM	C	KM	C	KM	SEM	Trt	T x P
Dry Matter Intake														
Total intake, kg/d	17.83	18.90	0.46	18.23	20.08	15.87	16.85	16.03	17.24	21.18	21.43	0.58	0.11	0.23
TMR intake, kg/d	8.22	8.08	0.15	6.69	6.41	7.06	6.73	6.90	6.86	12.24	12.32	0.17	0.49	0.58
Pasture intake, kg/d	7.95	9.02	0.38	9.89	11.87	7.12	8.41	7.48	8.45	7.32	7.35	0.51	0.06	0.12
Nutrient intakes														
OM, kg/d	16.33	17.26	0.41	16.78	18.02	14.37	15.62	14.69	15.67	19.46	19.74	0.36	0.13	059
N, kg/d	408.1	434.9	11.10	409.7	439.2	366.4	403.9	390.9	422.4	465.3	473.9	10.07	0.13	0.56
ADF, kg/d	4.98	5.30	0.14	4.95	5.42	4.09	4.56	4.10	4.42	6.79	6.82	0.13	0.14	0.37
NDF, kg/d	8.60	9.16	0.26	8.56	9.37	7.18	7.91	7.46	8.06	11.20	11.30	0.25	0.15	0.52
Nutrient digestibility														
DM, % of intake	69.32	69.00	0.23	71.66	71.31	69.45	68.60	69.09	68.84	67.08	67.24	0.32	0.34	0.20
OM, % of intake	70.57	70.18	0.27	73.30	72.12	70.38	70.22	70.53	70.02	68.05	68.37	0.37	0.34	0.06
CP, % of intake	64.61	63.51	0.44	63.88	61.76	66.18	66.49	65.67	63.73	62.70	62.04	0.72	0.10	0.11
ADF, % of intake	57.23	56.94	0.66	62.40	64.78	53.23	53.06	54.83	52.70	58.44	57.20	1.07	0.72	0.10
NDF, % of intake	62.64	62.34	0.43	66.90	66.25	59.82	59.62	62.29	62.14	61.56	62.14	0.44	0.60	0.97
ECM/DMI, kg/kg	0.81	0.80	0.04	0.89	0.87	0.89	0.79	0.84	0.89	0.62	0.63	0.04	0.83	0.29
FCM/DMI, kg/kg	0.74	0.74	0.04	0.84	0.82	0.82	0.74	0.76	0.81	0.56	0.57	0.04	0.86	0.34
Milk Yield/DMI, kg/kg	0.71	0.70	0.03	0.79	0.77	0.82	0.73	0.73	0.76	0.50	0.53	0.04	0.56	0.26

[1] Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$

Figure 1: Milk somatic cell count by treatment of lactating Jersey cows during the grazing season



APPENDIX A.

Institute for Animal Care and Use Committee Approval for Kelp Meal Winter Season Experiment (Chapter II)

University *of* New Hampshire

Research Integrity Services, Service Building
51 College Road, Durham, NH 03824-3585
Fax: 603-862-3564

16-Nov-2011

Brito, Andre Fonseca De
UNH Farms, Dairy T & R Ctr
Durham, NH 03824

IACUC #: 111002
Project: Feeding Kelp Meal to Organic Dairy Cows
Category: C
Approval Date: 19-Oct-2011

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category C on Page 5 of the Application for Review of Vertebrate Animal Use in Research or Instruction - *the research potentially involves minor short-term pain, discomfort or distress which will be treated with appropriate anesthetics/analgesics or other assessments.* The IACUC made the following comment(s) on this protocol:

Katherine McGeever and Brett Palmer need to complete the occupational health program for animal care personnel prior to handling animals on this study.

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. A Medical History Questionnaire accompanies this approval; please copy and distribute to all listed project staff who have not completed this form already. Completed questionnaires should be sent to Dr. Gladi Porsche, UNH Health Services.

If you have any questions, please contact either Dean Elder at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,

Robert C. Drugan, Ph.D.
Chair

cc: File
Whitehouse, Nancy

APPENDIX B.

Institute for Animal Care and Use Committee Approval for KM Grazing Season Experiment (Chapter III)

University *of* New Hampshire

Research Integrity Services, Service Building
51 College Road, Durham, NH 03824-3585
Fax: 603-862-3564

15-Jun-2012

Brito, Andre Fonseca De
UNH Farms, Dairy T & R Ctr
Durham, NH 03824

IACUC #: 120504
Project: Feeding Kelp Meal to Organic Grazing Dairy Cows
Category: C
Approval Date: 30-May-2012

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category C on Page 5 of the Application for Review of Vertebrate Animal Use in Research or Instruction - *the research potentially involves minor short-term pain, discomfort or distress which will be treated with appropriate anesthetics/analgesics or other assessments*.

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 Julie Simpson at 862-2003.

For the IACUC,
Jill A. McGaughy

Jill A. McGaughy, Ph.D.
Chair

cc: File
Whitehouse, Nancy