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Effects of ground flaxseed supplementation on animal production and milk fatty acid profile in organically-certified lactating Jerseys during the grazing season

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EFFECTS OF GROUND FLAXSEED SUPPLEMENTATION ON ANIMAL PRODUCTION AND MILK FATTY ACID PROFILE IN ORGANICALLY-CERTIFIED LACTATING JERSEYS DURING THE GRAZING SEASON

BY

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THESIS

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ABSTRACT

EFFECTS OF GROUND FLAXSEED SUPPLEMENTATION ON ANIMAL PRODUCTION AND MILK FATTY ACID PROFILE IN ORGANICALLY-CERTIFIED LACTATING JERSEYS DURING THE GRAZING SEASON

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Brianna J. Isenberg

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The objective of this thesis was to evaluate the effects of supplementing a pasture-based diet with ground flaxseed (GFLAX) on milk production and composition, blood parameters, digestibility, ruminal characteristics, nitrogen excretion, methane (CH_4) and carbon dioxide (CO_2) production, and income over feed cost (IOFC). Twenty organically-certified lactating Jerseys were blocked by milk production and days in milk (DIM) and randomly assigned to 1 of 2 treatments: 1) control (soybean meal and ground corn grain as 10% of total diet dry matter (DM) or 2) GFLAX as 10% of total diet DM. Treatments were top-dressed onto a 25% mixed grass-legume baleage, 23% grain meal, and 2% liquid molasses total mixed ration (TMR) (% of diet DM); pasture composed the remaining 40% diet DM. The study extended from June 8 to September 27, 2013 with 4, 28-d periods with the last 7 d used for data and sample collection. Dry matter intake, milk production, and milk component yields and concentrations were not affected by GFLAX

supplementation. Feed efficiencies, energy corrected milk, and 4% fat corrected milk did not differ between treatments. Body weight, body condition score, plasma nonesterified fatty acids, and serum cortisol showed no difference due to GFLAX supplementation. Apparent total tract DM digestibility was lower ($P = 0.04$) in cows on the 10% GFLAX treatment. Dietary treatment did not affect ruminal pH, individual or total volatile fatty acid concentrations. Cows receiving the 10% GFLAX diet had ($P < 0.01$) higher proportions of propionic acid in collected ruminal fluid. However, enteric CH_4 and CO_2 production did not differ between diets. Nitrogen intake ($P = 0.01$) and urinary urea N excretion ($P = 0.03$) were higher in cows on the 10% GFLAX diet due to higher crude protein concentrations of GFLAX. Milk fatty acid composition was altered by dietary flaxseed supplementation. Increases in concentrations of monounsaturated fatty acids ($P < 0.0001$) and n-3 fatty acids ($P < 0.0001$) with decreases in n-6 fatty acids ($P < 0.0001$) were detected in cows consuming 10% GFLAX creating a nutritionally enriched fatty acid profile in regards to human health. A lower IOFC was noted when GFLAX was included in the diet compared to the control ($4.61 vs. $5.53/cow/d, respectively), if premiums for nutritionally enriched milk are offered in the future, it may help offset the price differential.

INTRODUCTION

Growing interest in organically-produced food has contributed to an increase in organically-certified dairy production systems. While organic production composes only just over 4% of total U.S. food sales, dairy products are responsible for 15% of those sales (USDA-ERS, 2014). In 2008, the organic dairy industry sold over 1.25 million metric tons of milk (USDA-NASS, 2014). The 71% average annual increase in number of organic certified dairy cows reflects this trend with the national herd expanding from 38,000 cows in 2000 to over 254,000 cows in 2010 (USDA-ERS, 2014).

A survey completed by Hardie et al. (2014) indicated that Wisconsin organic dairies have a range of organizational and management structures. The surveyed farms were grouped into 1 of 4 categories, ranging from a large herd size, with heavy reliance on supplemental feed during the grazing season to a small herd size with seasonal calving using pasture and hay as the only forage source throughout the entire year. The results of this survey illustrate the diversity of organizational structures and practices adopted by organically-certified operations, emphasizing the need for additional research that can be directly related to improving management practices. Additionally, Wisconsin organic farms share similarities in size and structure with northeastern dairy farms (McBride and Greene, 2009).

Organically-certified dairies in the northeastern U.S. enable producers to meet a specific consumer demand. However, organic management practices can also present a set of challenges. In a survey of the needs of organic producers in the Northeast, Pereira et al. (2013) indicated that 79% of respondent farmers viewed balancing diets for energy

as a challenge, taking into consideration that 73.1% of participants are dependent upon purchased grains, which contribute approximately 36% of total cash expenses on farms in the northeast (Dalton et al., 2008). In addition, grain prices are subject to purchasing competition from other organic livestock sectors (Dalton et al., 2008). The same survey (Pereira et al., 2013) reveals 63% of respondents viewed additional focus on development of value-added products as an important research and educational need.

Supplementation with ground flaxseed (GFLAX; *Linum usitatissimum*) during the grazing season has a 3-fold benefit: 1) flaxseed is an oilseed high in energy, often a limiting component in pasture-based systems (Kolver and Muller, 1998), 2) flaxseed supplementation increases human health-promoting fatty acids in milk fat (Petit and Côrtes, 2010), and 3) flaxseed supplementation decreases enteric methane emissions (Beauchemin et al., 2009). Flaxseed may be used as an alternative to corn, however, during the period of current study, market prices for organic flaxseed and organic corn were $0.96 and $0.48/kg, respectively (USDA-AMS, 2014). The prices indicate organic flaxseed may be less available than organic corn. In addition, although flaxseed supplementation decreases enteric CH_4, it has also been reported to decrease diet digestibility (Scholljegerdes and Kronberg, 2010; Soder et al., 2012) and may be a concern for the current study.

The objective of this thesis was to supplement organically-certified lactating Jerseys with GFLAX during the grazing season to determine the effects on milk production, milk components, milk fatty acid composition, and ruminal metabolism. Additionally, the economic viability of supplementing GFLAX was considered in order to determine practical application for producers. In an assessment of operating costs by

the Agricultural Resource Management Survey of dairy operations (USDA-ERS, 2010), total gross value of production for organic operations in the Northeast was determined to be $30.82/cwt of milk sold. Average operating costs for these operations was assessed at $20.11/cwt, thus creating a profit of $10.71/cwt. These results emphasize the importance of cost-benefit analysis because results were widely varied based upon region. Briefly, guidelines and requirements of organically-certified dairies will be outlined, followed by a discussion on the use of pasture, including the challenges and effects of oilseed supplementation. Supplementing diets with GFLAX, and the subsequent effects on dairy cattle production, including milk fatty acid modification and methane production, will be explored. A brief overview of ruminal biohydrogenation under the influence of lipid supplementation will also be presented in the literature review.

CHAPTER I. REVIEW OF LITERATURE

Organic dairying

The term organic is defined as, "a labeling term that refers to an agricultural product produced in accordance with the [Organic Foods Production] Act and the regulations in this part." Organic agriculture is defined to be "a production system that is managed in accordance with the Act and regulations in this part to respond to site-specific conditions by integrating cultural, biological, and mechanical practices that foster cycling of resources, promote ecological balance, and conserve biological diversity" (USDA-NOP, 2014). All organic products are certified by a third party agency to ensure products meet the indicated standards. Any use of the term organic throughout this thesis is intended to be consistent with these definitions.

The USDA organic regulations are outlined in the Guide for Organic Livestock Producers (Coffey and Baier, 2012). A key portion of the guideline is that animals cannot be raised in a continuous total confinement operation. Specifically, ruminant animals must be maintained on pasture with daily grazing throughout the grazing season and have nearly continuous access to the outdoors throughout the year. The current grazing standard is a minimum of 120 days per calendar year with the goal of achieving not less than 30% of the dry matter intake (DMI) from grazing. The total feed ration must contain only organically produced and certified feeds. Urea, manure, mammalian and poultry

slaughter by-products, and antibiotics (including ionophores) are prohibited from diet formulations of organic animals (USDA-NOP, 2014).

An additional component of the organic requirements specifies animals may not be given growth-promoting drugs, including hormones. Animal health regulations are strict and prevent sale and labeling of any product as "organic" from an animal that has been treated with antibiotics. Although some vaccines are permitted, routine use of synthetic parasiticides is prohibited (USDA-NOP, 2014). Although both conventional and organic producers strive for preventative health management, the organic guidelines in place make preventative livestock health care a requirement in order to maintain a successful operation.

McBride and Greene (2009), using data from the annual Agricultural Resource Management Survey, categorized U.S. organic dairies based on size, region, and pasture utilization. Results of the survey indicated that over 80% of the organic dairies in the U.S. are located in the Northeast and Upper Midwest and are generally smaller and produce less milk at higher total economic cost per quantity of milk produced, than those found in the western U.S. Survey information also indicated that 45% of organic dairies milk less than 50 cows and 87% milk less than 100 cows. Farms in the Northeast have an average of 53 cows, while farms in the Upper Midwest have an average of 64 cows.

Pasture utilization

A survey of 987 farms across 4 northeastern U.S. states indicated approximately 13% of surveyed dairy farms use management-intensive or rotational grazing practices in

which the lactating herd is moved to a fresh pasture every 12 to 24 h (Winsten et al., 2010). Utilizing pasture as a forage source can be economically viable, and under the correct conditions, more cost effective than stored forage (Elbehri and Ford, 1995; Hanson et al., 1998). Although grazing systems may be less profitable than traditional forage systems (Parker et al., 1992; Tozer et al., 2003) due to a decrease in milk production that is often observed (Kolver and Muller, 1998; Agenäs et al., 2002; Bargo et al., 2002b), a corresponding decrease in DMI can create lower feeding costs than traditional forage systems (Tozer et al., 2003). Supplementation with additional forage (Phillips and Leaver, 1985) and concentrates (Stockdale et al., 1987; Stockdale, 2000, Bargo et al., 2002a), including fats (King et al., 1990; Schroeder et al., 2004), may alleviate some of the observed decrease in milk production. Supplementation with a total mixed ration (TMR) is also an option (Bargo et al., 2002b, Soder et al., 2003). An additional challenge of incorporating pasture into the ration is estimating DMI; a few available methods will be discussed later. Estimation of actual nutrient intake due to variability in dietary selection during grazing also presents a challenge.

Economics

Elbehri and Ford (1995) developed an economic simulation to evaluate the economic aspects associated with intensive grazing situations on typical Pennsylvania dairy farms. The Farm Level Income and Policy Simulator model was used with 10 farms, each with differing feeding systems; 6 utilized pasture while 4 did not. Each farm was structured to maintain 50 lactating cows, 10 dry cows, and 27 replacements as young stock. To mimic seasonal effects, 3 levels of pasture production were accounted for in the

model and each herd was grazed for 6 months. Results indicated that farms utilizing intensive grazing scenarios produced a net cash farm income of $140 to $207/cow per yr more than farms without intensive grazing. However, an assumption made with this simulation was all cows produced the same amount of milk, regardless of forage system. Two of the 6 grazing simulations remained more profitable than the non-grazing farms if milk production fell only 4-5%, comparatively. However, this analysis did not account for potential savings in ration costs that may be realized with the decrease in DMI that contributes to the fall in milk production.

Hanson et al. (1998) used two data sets to evaluate profitability of farms using moderately intensive grazing practices in the Northeast. The first data set used compiled results from a USDA study of New York and Pennsylvania farms while the second data set was based on 50 farms in Pennsylvania. In the USDA data set, moderate intensive grazing farms were defined as those in which cows received at least 15% of the forage from grazing, while extensive farms were those in which cows received less than 15% of the forage from grazing and included some confinement herds. Although the farms with moderate intensive grazing had 23% fewer cows with a 14% lower milk production per cow, they also received higher returns per cow than those farms with extensive grazing, $642 vs. $460, respectively, as well as a higher net farm income by $2,166. The higher net farm income of moderately intensive grazing systems was attributed to lower feed costs, lower cash expenses such as veterinary care, medicine costs, and hired labor, and lower overhead, interest, and capital investments.

The data set evaluated from the 50 Pennsylvania farms (Hanson et al., 1998) was separated based on moderate intensive grazing (cows moved to new pasture every 7 d or

fewer, pasture was ≥ 50% of herd forage needs for grazing season, and utilized more than 4 paddocks) and extensive grazing (farms that did not use all 3 parameters). In this data set, both moderate intensive and extensive grazing systems yielded higher net returns to management and owner equity when compared with corn silage and hay operations. While the moderate intensive grazing systems did have a significantly lower milk production per cow (9.5%) when compared to the extensive grazing systems, they also yielded a marginally higher profit ($61/cow). The evaluation of these data sets (i.e., the USDA and PA farms) indicates under the correct conditions, moderate intensive grazing systems can be profitable.

Parker et al. (1992) modeled both a traditional Pennsylvania dairy farm and a pasture-based farm. The pasture-based farm had a lower level of total operating expenses which translated to a $121/cow higher income compared with the traditional confined system. However, model inputs assumed average milk production was the same for both farms. Further evaluation indicates that overall income for the pasture-based dairy would not be higher than the confinement system if average milk production dropped by 450 kg/ lactation.

A whole farm analysis using the Dairy Forage System model was used to evaluate the economic impact of 4 levels of concentrate supplementation on a representative grazing dairy farm compared to a traditional confinement farm that did not use pasture but had similar land area, soil type and cow numbers (Soder and Rotz, 2001). The grazing farm used a spring seasonal calving strategy and animals were maintained on pasture all year, if pasture was unavailable or limited, animals were supplemented with hay or silage. In the model scenarios, the 4 levels of concentrate were provided at 0, 3, 6, or 9 kg

DM/cow/d, while annual milk production was set at 5000, 6068, 6968, and 7770 kg/cow, respectively, for each supplement level. Diet ingredients on the confinement farm included hay, silages, grain, and protein supplements and annual milk production was set at 9000 kg/cow. Additional factors that were taken into account included specific machinery, fertilizer, and labor costs associated with each farm. Production was estimated using 3 farm management scenarios: 1) all farms adjusted to similar milk production by increasing or decreasing animal numbers, 2) 100 mature animals on each farm, and 3) cow numbers are matched with forage production potential of each farm. When level of concentrate supplementation increased for the grazing farm, net returns to management increased, however, returns increased at a decreasing rate. The lower input costs associated with the grazing system indicated that the grazing systems with the high level of supplementation had a higher profitability compared to the confinement system despite a lower farm income.

A study conducted by Tozer et al. (2003) evaluated economic viability of 3 feeding systems: 1) TMR in confinement, 2) pasture with only a concentrate supplement, and 3) a partial TMR plus pasture (pTMR) diet. A partial budget analysis indicated that the pasture plus concentrate diet had the lowest feeding cost ($1.94/cow) followed by the pTMR ($2.70) and the TMR ($3.42). However, the pasture plus concentrate and the pTMR system experienced reduced milk production, 25% and 16%, respectively, when compared to the TMR system. The TMR system had the highest daily net income of $5.61/cow, followed by the pasture plus concentrate ($5.31) and the pTMR system ($5.28). The authors noted that the pasture used during the study was high in protein and the TMR used was identical for the TMR and pTMR systems. If the TMR had been

reformulated to reduce the protein and create a more realistic match with the protein supplied by the pasture, the net income for the pTMR system would increase to $5.53. Additionally, in both the pasture plus concentrate and the pTMR partial budgets, it was assumed that paddock fencing and water systems were not established and needed to be installed, creating a higher cost for these feeding systems. In both systems, costs associated with the fencing and water systems were allocated all at once and assumed to have an average use of 20 and 10 yr, respectively.

An evaluation of two grazing systems supplemented with either concentrate or TMR was compared with a similar sized confinement dairy feeding alfalfa and corn-based diets (Soder and Rotz, 2003). The Dairy Forage System Model was used to model the farm systems, and indicated that although the confinement system has the greatest profitability due to higher milk yield, the pTMR was only slightly less profitable than the confinement system. The grazing systems had lower production costs due to differences in machinery, storage, seed, and fertilizer costs as well as lower economic risk due to less reliance on corn yields.

Rotz et al. (2007) used the Integrated Farm System Model to evaluate 4 organic farms in PA as case studies and compared the results to conventional production practices. Under the conditions of the simulations, using 2005 market prices, organic management practices were noted as having an economical advantage over conventional production systems when production systems were scaled to a common land area, herd size, soil type, and weather conditions. However, the authors noted that the advantage is dependent upon organic milk prices and production per cow vs. organic and conventional systems.

Results from an Agricultural Resource Management Survey of U.S. organic farms (McBride and Greene, 2009) indicated that farms located in the Northeast and Upper Midwest have lower average feed costs per cow due to higher utilization of home-grown feeds and pastures. In addition, dairies that used more pasture had lower feed costs per cow. However, results indicated that decreased inclusion of pasture in the diet led to lower feed costs per unit of milk produced because average milk production per cow was 30% higher.

The results of Marston et al. (2011) agree with those of Rotz et al. (2007) and illustrate that even when similar feeding practices are followed on 2 separate farms, differing economic returns should be expected. Two separate herds, Holsteins at the University of Maine, and Jerseys at the University of New Hampshire, were maintained under organic management conditions for 3 consecutive years. The study used 4 diets; corn silage- or haylage-based supplemented with a complete concentrate pellet or commodity concentrate mix. Results of the study indicated that although feeding practices were the same, corn silage-based diets were significantly more costly than haylage-based diets at UNH, however, feed costs did not differ due to dietary treatment at the University of Maine. Although income over feed cost (IOFC) demonstrated numerical differences among diets at each location, diet did not significantly alter IOFC. The authors suggested the numerical difference between locations was due to differences in DMI as well as breed, confirming that profitability is related to many factors.

Hardie et al. (2014) surveyed 69 organically-certified Wisconsin dairy farms and classified the farms into 4 main operational structures or clusters detailed in the list below:

- Cluster 1 (n = 8) – Farms had larger herd sizes, used extensive feed supplementation during the grazing season, had a rolling herd average (RHA) production of 6,878 kg/cow/yr, and an IOFC of $10.17/cow/d.
- Cluster 2 (n = 5) – Farms were seasonal dairies that followed low-input methods, used only pasture and hay as forages during the grazing and non-grazing season, largely used breeds other than Holsteins, had an RHA production of 3,632 kg/cow/yr, and an IOFC of $5.76/cow/d.
- Cluster 3 (n = 32) – Farms utilized feed supplementation during the grazing season, however, were smaller in size than farms in cluster 1. These farms had an RHA of 7,457 kg/cow/yr and an IOFC of $8.59/cow/d.
- Cluster 4 (n = 24) – Farms were partially seasonal, moderate-input operations that utilized more pasture during the grazing season than farms in clusters 1 and 3. These farms had an RHA of 5,417 kg/cow/yr with an IOFC of $5.92/cow/d.

The lowest feed costs, for all 4 clusters, were observed during the grazing season when pasture was included in the ration. The authors suggested the higher RHA and IOFC in clusters 1 and 3 could largely be due to breed differences and feeding management practices. Although all the farms in each cluster used grazing practices, responses were varied based upon structural and managerial decisions.

Pasture feeding systems have widely varied economical results. The studies discussed illustrate that economic success is highly correlated to feeding and management strategies, including level of pasture inclusion in the diet, use of

supplementation, breed selection, and number of lactating cows. Although several of the studies have indicated that intensively grazed systems can be more profitable, calculating actual economic markers such as IOFC for each operation is a critical part of managerial assessment.

Production

When high producing Holstein cows were fed either TMR or pasture only, a 19% decrease in DMI was observed on the pasture diet (Kolver and Muller, 1998). The decrease in intake translated to a 33% reduction in milk production from the cows on pasture compared with those receiving the TMR diet. In addition, cows on pasture experienced higher rates of body weight (BW) and body condition score (BCS) losses. Mobilization of body fat was higher in grazing cows, as indicated by higher blood concentrations of beta-hydroxybutyrate and nonesterified fatty acids (NEFA). Cows on the pasture-only diet had a DMI of 3.39% of BW compared with those on the TMR diet with a DMI of 3.93% of BW. The limited DMI directly depressed milk production, specifically by failing to provide an adequate supply of energy. Pasture and TMR provided similar concentrations of energy when expressed as net energy of lactation, however, pasture was slightly higher than TMR, 1.65 vs. 1.63 Mcal/kg. This further indicates that intake limited energy rather than availability of energy in the diet. Agenäs et al. (2002) also observed a significant decrease in milk production and BW when cows were turned out to pasture. In the same study, plasma insulin decreased and NEFA values increased at pasture turn-out suggesting that limited nutrient intake minimized production potential.

Supplementation during the grazing season can be used to optimize production while maintaining pasture in the ration. Phillips and Leaver (1985) conducted 2 studies in which a pasture-only diet served as the control in each experiment. In experiment 1, pasture formed the basal diet while the 3 treatment groups were offered 45 min of grass silage at a rate to achieve 10% orts, restricted grass silage overnight (4 kg DM/d), or ad libitum grass silage overnight at a rate to achieve 10% orts. In experiment 2, pasture again formed the basal diet while the 2 treatment groups were offered 45 min of grass silage or ad libitum grass silage overnight both at a rate to achieve 10% orts. In experiment 1, milk yield was decreased by offering silage, however, milk production was increased in experiment 2. Although the energy concentration of pasture was higher than that of the silage, the decrease in milk yield observed in experiment 1 is likely due to lower vitro digestible organic matter of the silage compared with pasture because energy intake was similar among diets. Silage supplementation also increased rumination time, milk fat concentration and milk fat yield, further indicating that fiber content contributed to differences in milk production in experiment 1. Grazing conditions were less favorable during experiment 2 than experiment 1 and silage supplementation increased dietary energy intake when compared to the pasture-only diet. The increase in energy intake from silage supplementation contributed to increases in milk production. In addition, fluctuations in daily mean milk production were minimized by silage supplementation through maintenance of DMI.

In 5 experiments, Stockdale et al. (1987) fed different levels of pelleted, high energy barley or wheat-based supplement to complement a basal ration of forage-harvested pasture for stall-fed dairy cows. Results showed a positive linear relationship

between milk production and pellet intake in each experiment. For each additional kilogram of pellets consumed, 0.7 to 1.8 kg of milk was produced. The marginal return tended to be higher early in lactation and decreased later in lactation. The decrease in milk production response is consistent with the law of diminishing returns in which each additional increase in input results in smaller increase in output. The authors attributed the diminishing returns to decreased digestibility as pellet level increased in the diet and this was supported by increased fecal starch. Although milk fat concentration tended to be depressed with higher pellet intake, higher milk fat yield was observed. The higher milk fat yield may be attributed to higher milk production that created a dilution effect, thus decreasing milk fat concentration.

Reis et al. (2000) reported increases in milk production when grazing cows were provided with a ground dry shelled corn supplement at 0, 5, and 10 kg of DM/d. Cows in the study grazed for approximately 20 h/d. Cows consuming 5 and 10 kg of DM/d of supplement produced 18.7 and 28.3% more milk than cows on the pasture only treatment. A linear increase in DM and organic matter (OM) intake and digestibility was noted when cows consumed increasing levels of grain supplement, however, pasture DMI was decreased by supplement consumption. This effect of decreased pasture intake in response to supplementation is known as substitution rate.

Stockdale (2000) conducted 2 experiments. In experiment 1, cows were divided into low BCS or high BCS groups and assigned to a pasture-only or a pasture supplemented with a pelleted barley/wheat-based (75 and 25% of DM, respectively) mixture at a rate of 5 kg DM/d. Cows receiving the supplement demonstrated an increase ($P = 0.05$) in milk production and maintained BCS. In experiment 2, supplementation

with the same pelleted barley/wheat mixture also increased ($P = 0.05$) milk production of cows that were assigned to high or low BW groups while on pasture. In addition, supplementation promoted BW gain during the study.

Bargo et al. (2002a) provided a dry shelled corn-based concentrate (at a rate of 1 kg concentrate/4 kg milk production) to cows grazing at a high or low pasture allowance. Cows were assigned to 1 of 4 treatments: 1) low pasture allowance and no supplement, 2) low pasture allowance plus supplement, 3) high pasture allowance and no supplement, and 4) high pasture allowance plus supplement. Cows receiving the corn supplement demonstrated increased milk production, with greater increases observed in cows on the low pasture allowance treatment. Low pasture allowance may have limited DMI because higher pasture intake ($P < 0.01$) was observed when pasture allowance was higher. In agreement with Stockdale et al. (1987), supplementation decreased milk fat concentration, but increased milk fat yield for both groups receiving concentrate. Although supplementation did not affect BW or BCS, NEFA values were elevated for unsupplemented cows. Elevated NEFA values suggest that unsupplemented cows were mobilizing more body fat than their supplemented counterparts.

King et al. (1990) evaluated a barley-based concentrate supplement on performance of grazing cows. The control group consumed a pasture-only diet, a second group grazed and received the barley-based concentrate (3.3 kg/d) without added free fatty acids (FA), and the third group grazed and received the barley-based concentrate (3.8 kg/d) with added free, long-chain FA (mostly palmitic, stearic, and oleic acid). Concentrate supplementation tended to increase milk production in both groups compared with the pasture-only group. A trend for higher milk production was observed

when additional FA were included in the supplement in comparison to the group receiving concentrate with no added FA.

Bargo et al. (2003) reviewed the effects of supplementation on animal production parameters for dairy cows on pasture. The review indicated that supplementing cows on pasture decreased time spent grazing which may account for the decrease in pasture DMI, thus contributing to the substitution rate. However, total DMI was typically increased by supplementation. Studies included in the review indicated that supplementation increased milk production by an average of 4.4 kg/d when compared with cows consuming a pasture-only diet. Concentration of milk fat was reduced in several studies when concentrates were included in pasture diets, whereas milk fat yield increased in a number of studies. When the effects of supplementation of fat were evaluated in relation to milk production, the studies included in the review showed inconsistent results. While some studies showed no effect on milk production, others resulted in positive effects.

A review by Schroeder et al. (2004) summarized several studies in which cows on pasture had been supplemented with fat sources. The review suggested that supplying fat to cows on pasture would be a beneficial way to meet energy requirements and observed that fat supplementation increased milk production by 4.5% when supplied to grazing cows. Potential disadvantages of supplementing grazing diets with fat included modification of ruminal function leading to decreased fiber digestion and possible depression of milk fat production.

Three feeding systems were evaluated by Bargo et al. (2002b). Cows were assigned to 1 of 3 treatment groups: 1) TMR, 2) pasture plus supplemental concentrate, or 3) pTMR. Relative to the TMR treatment group, milk production was reduced in both

grazing treatments. However, the pTMR treatment led to additional 3.5 kg/d of milk production compared with pasture plus concentrate.

Rego et al. (2008) evaluated the effects of supplementation using 4 different feeding strategies: 1) grazing 20 h/d with 6 kg/d of a corn-based concentrate, 2) grazing 20 h/d with 6 kg/d of a corn soybean meal-based concentrate, 3) grazing 7 h/d with 6 kg/d corn-based supplement and 13 h/d ad libitum grass silage at night, and 4) grazing 7 h/d with 6 kg/d corn soybean meal-based supplement. Total DMI was not affected by silage supplementation, however, an interaction between silage supplementation and concentrate supplementation suggests that soybean inclusion coupled with grass silage supplementation led to higher DMI. Milk yield and energy corrected milk (ECM) did not differ among all 4 treatments, however, soybean meal inclusion tended to increase milk yield. The authors suggested that silage supplementation at night may be a useful alternative during times of reduced pasture availability.

Although results of supplementing pasture with concentrates, fats, or forages are quite variable, the overall trends demonstrate improved total DMI and milk production. If supplementation can be achieved in a cost efficient way, it could make pasture-based feeding a more attractive option for producers interested in alternative management techniques.

Estimation of pasture intake

Determining total DMI for cows grazing pasture presents a challenge, specifically, estimating pasture DMI. Bargo et al. (2003) reported that a significant disadvantage of estimating pasture intake via pasture measurement methods (i.e., pre- and

post-grazing herbage mass) is that the method provides group intakes rather than individual cow intakes. Another option for estimating intake is dosage with an external marker like chromium oxide or alkanes to estimate fecal production and diet digestibility.

Macoon et al. (2003) evaluated 3 methods for estimating pasture intake of grazing dairy cows. The 3 methods used were: 1) inference from animal performance, 2) evaluation from fecal output using a pulse-dose marker, and 3) estimation from herbage disappearance methods. Animal parameters such as average daily BW gain and energy expended walking to pasture were used to calculate net energy of lactation and used to estimate forage intake. In the pulse-dose method, animals were dosed with chromium mordanted fiber and the concentration in feed and feces was used with digestibility parameters to estimate fecal output. The herbage disappearance calculated the difference between pre-grazing and post-grazing herbage mass to determine forage intake. Inference from animal performance and herbage disappearance were positively correlated and led to similar estimates of DMI. A challenge of the animal performance method is individual animal characteristics may be lost because general equations are used in the calculations. The pulse-dose method estimated higher forage DMI and a portion of the difference may be due to the limitation in which accurate estimates can only be made when the marker is being dosed and samples collected, rather than being projected onto the entire experimental period.

Smit et al. (2005) compared 3 techniques to estimate pasture intake in grazing dairy cows during 2 grazing seasons. The first technique measured the difference between pre- and post-grazing herbage mass. In the second method, cattle were dosed with an even-chain n-alkane that served as a fecal marker. Grasses are high in odd-chain

length alkanes and the amount of alkane contained in the sampled herbage can be used in combination with the ratio of dosed and naturally occurring alkanes in the feces to determine herbage intake. In the third method, DMI was calculated based on animal parameters such as cow energy requirements for lactation and maintenance and energy intake from the grazed forage. The pre- and post-grazing method demonstrated the highest coefficient of variation and was not consistent between study years. Using n-alkanes produced results with less variation and the authors concluded that n-alkanes are the preferred way to estimate pasture intake among the three evaluated methods.

Ferreira et al. (2004) also used n-alkanes and chromium oxide in controlled-release capsules to estimate feed intake. The cattle were individually fed a diet of meadow hay only and dosed with either n-alkane or chromium oxide capsules. The concentration of the selected n-alkanes in the collected fecal samples and meadow hay, as well as the capsule release rate, were used to calculate estimated DMI. Diet digestibility, measured as acid insoluble ash (AIA) and fecal output (calculated using the release rate of the marker and the concentration in the collected feces) were used to estimate forage intake when cows were dosed with chromium oxide. No significant differences between the actual DMI and estimated DMI were noted when n-alkanes were used, however, based on the chain-length of n-alkane selected for analysis, a 10 to 15% overestimation in DMI was observed. Estimation using chromium oxide and AIA as a digestibility marker did not differ from the measured DMI.

Chromium oxide may be used in combination with in vitro dry matter digestibility (Holden et al., 1994; Detmann et al., 2001) and indigestible acid detergent fiber (Detmann et al., 2001) to estimate intake. Detmann et al. (2001) used multiple methods to

evaluate pasture intake in crossbred steers. When chromium oxide was used in combination with in vitro dry matter digestibility, estimated DMI was higher (3.16% of BW) than that calculated using indigestible acid detergent fiber (2.72% of BW). However, 1 limitation of using in vitro dry matter digestibility is the assumption that all cows have the same level of digestibility.

Although several methods for estimating pasture intake may be used, each method offers a set of benefits and challenges. Careful consideration and use of multiple methodologies may be appropriate depending upon the measurements required for each situation.

Flaxseed supplementation

To date, no studies have evaluated supplementing pasture with GFLAX. However, 1 study replaced orchardgrass with GFLAX in dual flow continuous culture (Soder et al., 2012), 3 evaluated the effects of extruded flaxseed on grass-based diets (Lerch et al., 2012a; Lerch et al., 2012b; Lerch et al., 2012c), and several evaluated flaxseed, in various forms, as a supplement for lactating cows in confinement (Gonthier et al., 2005; Bell et al., 2006; da Silva et al., 2007; Petit and Côrtes, 2010; Chilliard et al., 2009; Neveu et al., 2013). The effects of flaxseed supplementation on production will be discussed below.

Gonthier et al. (2005) reported a non-significant decrease of 1.8 kg of milk production per d observed in cows consuming ground, raw, micronized, or extruded flaxseed compared with the control diet without flaxseed. The diets were formulated to contain flaxseed at 12.6% of DM and treatment did not affect DMI. Flaxseed

supplementation did not alter concentration of milk fat, protein or lactose. However, milk protein yield was reduced when cows received flaxseed compared with the control diet.

Bell et al. (2006) did not observe a shift in milk production or DMI between treatments when flaxseed oil was included in the diet at a rate of 6% of DM (in combination with 150 IU vitamin E/kg of DM). A significant decrease in milk fat concentration was noted between wk 0 and 2, however, milk fat concentration in cows receiving flaxseed oil plus vitamin E was recovered to the original level of production by wk 4 and maintained throughout the remainder of the study.

A trend for higher milk production (+6.5%) was observed when cows were supplemented with GFLAX (12% of DM) compared with whole flaxseed (12% of DM) (da Silva et al., 2007). Although 4% fat corrected milk (FCM) did not differ between GFLAX or whole flaxseed treatments, a slight trend for lower milk fat concentration was observed when cows received GFLAX as opposed to whole flaxseed.

Flowers et al. (2008) reported no difference in milk production when grazing cows consumed a cracked corn and soybean meal-based supplement containing added flaxseed oil. Cows in the control group received the supplement but no flaxseed oil while the cows in the 3 treatment groups received the supplement with 170, 340, or 510 g/d of added flaxseed oil. Although milk production was not affected, milk fat and protein concentrations showed quadratic increases.

Chilliard et al. (2009) fed 4 corn silage-based diets: 1) a control diet without flaxseed, 2) whole flaxseed, 3) extruded flaxseed, and 4) flaxseed oil. Flaxseed was added to the diet to compose 5% of diet DM and replaced a portion of the concentrate. Milk yield of cows consuming flaxseed oil was significantly lower than cows on the other

3 diets. In addition, yields of milk fat, protein, and lactose were significantly lower when cows consumed extruded flaxseed and flaxseed oil compared with the control diet.

A decrease in DMI was observed when cows were fed a GFLAX diet (7.2% diet DM) compared to a control diet with calcium salts of palm oil, a diet supplemented with whole flaxseed (7.2% diet DM), or a diet supplemented with an even mixture of whole flaxseed and GFLAX (Petit and Côrtes, 2010). The observed decrease in DMI did not translate to decreased milk yields, resulting in similar production for all diets throughout the study. Milk fat and milk protein concentrations and yields were not affected by dietary treatments.

A long-term study by Lerch et al. (2012a) investigated supplementation of canola seed or flaxseed in grass-based diets for 2 consecutive lactations. At the start of lactation, cows were housed indoors and received a basal diet of 75% grass silage and 25% grass hay ad libitum. Cows were gradually adjusted to pasture over the period of a week and eventually had access to grazing 20 h per d. Oilseeds were included in the diet to achieve a rate of 2.5 to 3.0% additional oil in ration DM compared with the control diet during both the indoor and outdoor periods. Treatment groups included extruded flaxseed, extruded canola, cold-pressed fat rich canola meal, or whole, unprocessed canola seeds. In the first year of the experiment (indoor and outdoor period), no significant effects of oilseed supplementation were observed on milk production. No effects were observed on fat, protein, or lactose yields when cows receiving extruded flaxseed were compared with those on the control treatment. Compared with the control diet, a trend for decreased forage DMI during the indoor period was observed in cows supplemented with oilseeds. Body weight and BCS were not affected by treatments during indoor or outdoor periods.

Results from the second year of the experiment (Lerch et al., 2012a) were reported comparatively to the first year to evaluate within-cow differences. Milk production increased significantly in the second year of the study, however, the increase was not due to dietary treatments and the observed increases did not differ between the control and flaxseed supplemented groups. Although cows receiving extruded flaxseed demonstrated decreased concentration of milk protein from year 1 to year 2 during the indoor and outdoor periods, the change was not different from that observed in control cows. Milk fat concentration also decreased during the indoor period and showed a slight increase during the outdoor period, but it was similar to the results obtained with the control cows. Milk protein and milk fat yields were increased during the indoor and outdoor periods of year 2 compared with year 1 for both control and extruded flaxseed supplemented cows, but there were no differences between the 2 treatment groups.

When extruded flaxseed was fed or not as a part of a high (60%) or low forage (40%) diet, milk production was not different between treatments (Neveu et al., 2013). Energy corrected milk, 4% FCM, and solids corrected milk production were all higher when cows received a high forage diet, but were not affected by flaxseed supplementation.

The studies summarized above illustrate various responses to flaxseed supplementation, under various feeding and management strategies. The variation indicates that although positive production responses can be achieved, they are influenced by many factors including flaxseed processing, composition of the basal diet, and level of flaxseed inclusion. Additionally, because no studies have been conducted using GFLAX

during the grazing season, research in this area could provide useful results for farms evaluating GFLAX as a feeding option.

Milk fat depression

Several of the studies previously cited indicated that pasture feeding, concentrate supplementation, and flaxseed feeding can lead to decreased milk fat content. A review by Bauman and Griinari (2003) indicates that decreased milk fat can be separated into 2 broad categories: 1) diets that provide large amounts of readily digestible carbohydrates and reduced amounts of fibrous components and 2) diets supplemented with polyunsaturated fatty acids (PUFA). The review suggests 3 theories to explain the decrease in milk fat content. The underlying principle is that feeding either of the 2 categories of diets alters ruminal function and microbial processes. The first suggested mechanism is that feeding increased levels of concentrate and lower levels of forages leads to decreased ruminal acetic and butyric acid production. Acetic and butyric acid are major building blocks used for milk FA synthesis in the mammary gland. In addition, rapidly fermentable substrates may cause a low ruminal pH and unfavorable conditions for ruminal cellulolytic bacteria, which are the main producers of acetic and butyric acid. Although pasture provides fiber, high quality pasture is rapidly digestible and may not provide an adequate amount of slowly degradable fiber to maintain steady ruminal pH by promoting rumination and saliva production, which provides a buffering effect.

The second suggested mechanism (Bauman and Griinari, 2003) is an increase in propionic acid production that leads to up-regulated hepatic gluconeogenesis rates and

insulin rates, creating competition for substrates in the mammary gland. The theory indicates that increased circulating insulin levels may direct nutrients such as acetate, β-hydroxybutyrate, and diet-derived long-chain FA, which are substrates for milk FA production to non-mammary tissues.

The third proposed mechanism (Bauman and Griinari, 2003) suggests that ruminal function is altered by dietary supplementation with unsaturated FA and creates intermediate FA products that inhibit milk fat synthesis. An example of an intermediate that can be produced is *trans*-10, *cis*-12 CLA which can cause a 25% reduction in milk fat yield when dosed at 3.5 g/d (Baumgard et al., 2001). The fat contained in pasture and flaxseed is high in unsaturated FA (NRC, 2001; Schroeder et al., 2004) and could contribute to modification of ruminal processes.

It should also be noted that although some studies reported decreases in milk fat concentration, an increase in milk fat yield was also observed as indicated in a review of supplementation of pasture cows by Bargo et al. (2003). This suggests that an increase in milk production created a dilution effect that led to decreased milk fat concentration, but overall increase in milk fat yield.

Ruminal function and biohydrogenation

Crude fat composes 3 to 8% of fresh pasture DM, with 1 to 3% FA in temperate pastures (Schroeder et al., 2004). Fat concentration in pasture varies based upon species, maturity, and season (Bauchart et al., 1984). Less mature plants during the spring and fall have higher fat concentration and a shorter time between cuttings will also increase fat

levels. The fat found in pasture is mostly unsaturated and 2 of the main FA are linoleic (C18:2) and linolenic (C18:3) acids. Flaxseed is also high in unsaturated FA containing 53 g linolenic/100 g FA and 12.7 g linoleic/100 g FA (NRC, 2001).

Ruminal microbes modify feeds to a great extent after they have been ingested. When unsaturated fats, such as those found in pasture and flaxseed, enter the rumen, microbes begin to metabolize the fat. Ruminal modification of fat is also called biohydrogenation; this is the process where double bonds are replaced by hydrogen to convert unsaturated FA to saturated FA (Jenkins and McGuire, 2006). As lipids enter the rumen, microbial lipases hydrolyze the ester linkages to release FA (Jenkins et al., 2008). Once the FA have been released, they are isomerized by the ruminal microbes, generally changing the conformation of the double bond from *cis*- to *trans*-FA (Chilliard et al., 2000). Following isomerization, the FA are hydrogenated to remove double bonds with the most common end product being C18:0 or stearic acid.

In the biohydrogenation of linoleic acid, it enters the rumen as *cis*-9, *cis*-12 C18:2 and no *trans* double bonds. Bacterial isomerases alter the double bond configurations to include one or more *trans* double bonds, creating *cis*-9, *trans*-11 C18:2, or conjugated linoleic acid (CLA). Bacterial reductases then desaturate the double bonds to yield *trans*-11 C18:1 and a second reduction hydrogenates the FA to form the final product stearic acid (Chilliard et al., 2000; Jenkins and McGuire, 2006). The biohydrogenation path for linolenic acid is similar to that of linoleic acid, however, more steps are required due to its 3 double bonds (Lourenço et al., 2010). The rate-limiting step is the conversion of *trans*-11 C18:1 to C18:0. Due to the high concentrations of linoleic and linolenic acid in flaxseed and fresh pasture, this could lead to an accumulation of the biohydrogenation

intermediate *trans*-11 C18:1, increasing the amount that passes out of the rumen to be absorbed in the small intestine (Schroeder et al., 2004). An average of 80 and 92% of linoleic and linolenic acids, respectively, are biohydrogenated (Chilliard et al., 2000), however, the rate and extent can be influenced by diet, type and amount of fat, and ruminal pH (Jenkins et al., 2008).

Ruminal biohydrogenation when cows consume forages may also vary based upon botanical species. In a review by Lourenço et al. (2008), red clover vs. ryegrass-based diets led to higher linolenic acid flows and ruminal proportions in most studies. When red clover diets were compared to white clover diets, a tendency for lower linolenic acid apparent biohydrogenation was reported. Lower biohydrogenation of linolenic acid is associated with the plant metabolite polyphenol oxidase (PPO) found in red clover. The metabolite has 3 proposed mechanisms: 1) lower plant lipase activity, 2) inhibition of microbial lipases, and 3) phenol bound proteins that protect the plant FA from lipases (Lourenço et al., 2008). A final comparison revealed that grass-based diets produced lower proportions of C18:1 and CLA when compared to botanically diverse diets that included herbs.

Volatile fatty acid production

Ruminal volatile fatty acids (VFA) production is altered based upon dietary parameters, including supplementation of fats (Schroeder et al., 2004), supplementation of concentrates (Stockdale et al., 1987), and pasture consumption (Bargo et al., 2003).

Total ruminal VFA production was not significantly different when cows were supplemented with micronized, extruded, or crushed flaxseed (Gonthier et al., 2004;

Beauchemin et al., 2009; Neveu et al., 2013; Neveu et al., 2014). However, molar proportions of VFA showed varying results. Gonthier et al. (2004) reported that diets containing micronized or extruded flaxseed decreased molar proportions of acetic acid and increased molar proportions of propionic acid relative to control diets. When crushed flaxseed was included in the diet, only butyric acid concentration was lowered (Beauchemin et al., 2009). Extruded flaxseed supplementation did not influence molar proportions of any reported VFA (Neveu et al., 2013). The molar proportion of acetic acid was not affected by extruded flaxseed supplementation (Neveu et al., 2014). However, lower propionic acid and higher butyric acid molar proportions were noted with flaxseed consumption.

Scholljegerdes and Kronberg (2010), using beef heifers, fed a pasture-only diet as the control along with 2 treatments: 1) grazing plus a corn/soybean meal concentrate at 0.32% of BW once daily and 2) grazing plus GFLAX at 0.18% of BW once daily. A trend for decreased ruminal VFA production was observed when cattle were supplemented with either concentrate. A decrease in molar proportion of acetic acid was observed in supplemented cattle and the greatest decrease was noted in cattle consuming GFLAX. Molar proportions of propionic acid were not significantly different between diets, however, butyric acid proportions were increased in supplemented cattle and the greatest increase was in the corn/soybean meal-based supplement.

Soder et al. (2012) used continuous culture fermenters to evaluate supplementation of an orchardgrass-based diet with 0, 5, 10, or 15% of DM replaced with GFLAX. Ground flaxseed treatments did not change total VFA concentration, however, an increase in molar proportions of acetic and propionic acid was observed with GFLAX

inclusion. Additionally, GFLAX decreased molar proportions of butyric and valeric acids.

Ruminal VFA results have demonstrated a wide range of responses that are sensitive to changes in diet. The wide variation in responses indicates that effects on ruminal VFA should be evaluated when possible in diets supplemented with flaxseed.

Methane production

Methane (CH_4), and carbon dioxide (CO_2) are by-products of ruminal fermentation (Moss et al., 2000). Hydrogen, in the form of H_2, is removed from the rumen via production of CH_4. Methane is a potent greenhouse gas and the U.S. EPA (2012) has estimated that animal agriculture contributes 3.8% of national production. Methane production is an environmental concern as well as a production concern. In fact, CH_4 production represents a loss of 5 to 7% of gross energy to the animal (Hristov et al., 2013). Methane is produced in the anaerobic conditions of the rumen and the process is carried out by methanogens called archaea. Although production can vary based upon DMI and animal size, the average cow produces 60 to 160 kg CH_4/yr (Hristov et al., 2013). High forage diets, especially those with low digestibility, lead to increased CH_4 production, 6 to 7% vs. 2 to 3% of energy intake, when compared with high grain concentrate diets, respectively (Moss et al., 2000).

Hristov et al. (2013) determined that grazing management has a CH_4 mitigating potential of less than 10%, however, this strategy can be effective if higher quality pastures are maintained. Supplementing ruminant diets with fat has been investigated as a method of reducing CH_4 production. A review by Hristov et al. (2013) has rated lipid

supplementation an effectiveness level of "medium" for mitigating CH_4 emissions. This translates to a 10 to 30% mitigating effect when compared to standard practice. In addition, the authors do recommend the use of dietary lipids for mitigation, when economically viable, because it has been deemed effective and is safe for both the environment and the animal. The proposed mechanism for decreasing CH_4 is by decreasing DMI with a combined increase in milk production, which results in an increased feed efficiency. A second proposed mechanism is that unsaturated fats serve as a method for eliminating H_2, however, this contribution is expected to be small (Jenkins et al., 2008). In addition, supplemental fats also decrease the amount of OM digested in the rumen and lower the activity and functionality of ruminal archaea and protozoa (Beauchemin et al., 2009).

The effects of flaxseed supplementation on CH_4 output were evaluated by Martin et al. (2008). The control diet was used as the basal diet for each of the 3 treatment groups. Treatments included whole flaxseed, extruded flaxseed, and flaxseed oil, and they replaced a portion of the concentrate in the basal diet at a rate to achieve a theoretical level of 5% oil of diet DM. Methane production was measured for 5 days using the sulfur hexafluoride (SF_6) technique. A significant difference was detected for all diets when comparing CH_4 production. Cows on the control diet had the highest CH_4 emissions followed by whole flaxseed, extruded flaxseed, and flaxseed oil. The same was true when CH_4 was expressed as g/kg OM intake or as a percentage of gross energy intake. Although cows receiving flaxseed (whole, extruded, or oil) did demonstrate decreases in DMI, a 7% reduction in neutral detergent fiber (NDF) digestibility was likely the main cause of reduced CH_4 production.

Calorimetric chambers were used to measure the CH_4 emissions of cows supplemented with varying sources of long-chain FA (Beauchemin et al., 2009). The control diet contained calcium salts of long chain FA while the 3 treatment diets consisted of crushed sunflower seed, crushed flaxseed, or crushed canola seed. Oilseeds provided a range of 3.1 to 4.2% additional fat to diet DM. Cows receiving the flaxseed supplement demonstrated an 18% decrease in CH_4 production (g/cow/d) compared with the control diet. However, g CH_4 production per kg digestible DMI was not different between control or flaxseed supplemented cows.

In vitro work with GFLAX indicates that supplementation does decrease CH_4 production in conjunction with pasture (Soder et al., 2012). Freeze-dried orchard grass in vegetative stage was used to feed continuous culture fermenters along with GFLAX at 0, 5, 10, or 15% of total DMI. Methane production demonstrated a linear decrease as flaxseed increased in the diet, however, linear decreases in apparent DM, OM, and NDF digestibilities were also observed. The decrease in diet and nutrient digestibility may limit the practical application of flaxseed in CH_4 mitigation.

Resende et al. (2013) evaluated supplementation of GFLAX on CH_4 production using 20 cows consuming GFLAX at 0, 5, 10, or 15% of diet DM. Methane production was measured using the SF_6 tracer technique. A significant linear decrease (285, 262, 259, and 225 g CH_4/cow/d) was observed when cows were fed increasing levels of flaxseed 0, 5, 10, and 15% of diet DM, respectively. However, a significant linear decrease in DMI translated to reduced milk production, indicating that higher levels of flaxseed may have impaired ruminal digestion.

Milk fatty acid composition

Potential human health benefits

Interest in modifying food products to contain more human health-promoting components has been increasing over the years. Modification of the bovine milk FA profile has received a great deal of attention and many attempts have been made to shift the FA to a profile more favorable for human health. Ip et al. (1994) documented results that indicated feeding CLA (i.e., *cis*-9, *trans*-11 and *trans*-10, *cis*-12) at incremental levels of 0.05, 0.10, 0.25, and 0.50% of the basal diet inhibited mammary carcinogenesis in a dose-dependent relationship in rats that had been administered a known carcinogen. It was also observed that feeding a diet containing 1% CLA for 5 wk (weaning to 50 d; the period of mammary gland maturation in rats) prior to carcinogen dosing provided a protective effect and decreased tumor development 34 to 39% (Ip et al., 1994). The authors provided an extrapolation in which 0.10% CLA in the diet was proposed as the most effective dosage, translating to a daily CLA intake of 3 g/d for a 70 kg human to obtain protective benefits.

Ritzenthaler et al. (2010) used 3 methods to estimate relative intakes of CLA in men and women aged 18 to 60. Based upon these results, average CLA intake was less than 500 mg/d for both genders and all age groups. The study also indicated that dairy products are the major source (60%) of CLA consumed in the diet, with cheese being the biggest contributor. Using milk fat concentration and CLA concentration, an estimate of average consumption of CLA per serving of milk (250 g) can be calculated. When the results of Lerch et al. (2012b), in which cows consumed extruded flaxseed during the

grazing season were used, average CLA consumption per serving of milk was calculated to be 160 mg CLA. Resende et al. (2013) fed GFLAX (15% of diet DM) to cows consuming a TMR and observed 88 mg CLA per serving of milk. An additional consideration is that low-fat dairy products contain less fat which translates to lower levels of CLA provided with each serving of dairy. Based on the results of Lerch et al. (2012b) and Resende et al. (2013), in order to achieve the suggested dietary intake of 3 g CLA/d, an individual would need to consume 19 or 38 servings, respectively, of whole milk per day.

Omega-6 (n-6) and omega-3 (n-3) PUFA are essential FA that must be included in the human diet and are found in low levels (Kennelly, 1996) in bovine milk. The American Dietetic Association (Kris-Etherton et al., 2007) recommendations for intake, as based on a 2,000 calorie diet, suggest 7 to 22 g/d of n-6 and 1.8 to 3.2 g/d of n-3 PUFA. n-3 FA are associated with health benefits for humans (Simopoulos, 2002) while high intakes of n-6 FA, as commonly found in Westernized diets, are associated with increased production of proinflammatory agents. n-3 FA may have anti-inflammatory effects, decreasing incidence of cardiovascular disease, rheumatoid arthritis, diabetes, and inflammatory bowel disease (Simopoulos, 2002). Increased intake of n-3 FA while decreasing intake of saturated FA is also recommended (Vannice and Rasmussen, 2014).

A survey of 500 customers indicated that 98% of respondents were aware of health benefits of n-3 FA and n-3 enrichment of products would have a substantial impact on food purchasing decisions (Feedstuffs Foodlink, 2014). Dairy products contribute the majority of CLA in the diet as well as a portion of the n-3, n-6, and saturated FA,

providing an excellent opportunity to modify milk FA composition in an attempt to provide a more healthful FA profile with each serving of dairy.

Milk fatty acid synthesis

The main components of milk fat are triglycerides, which compose 96 to 99% of the lipids, with phospholipids and sterols composing less than 1 and 0.5%, respectively (Timmen and Patton, 1988). Milk fat synthesis can be separated into 2 general pathways: 1) FA synthesized in the mammary gland, or *de novo*, and 2) those arising from the diet or body stores. Short-chain FA (C4:0 to C8:0) and medium-chain FA (C10:0 to C14:0) are synthesized *de novo*, C16:0 FA may be synthesized *de novo* or originate from the diet, while long-chain FA (> C16:0) are attributed to dietary sources or body stores (Bauman and Griinari, 2003).

De novo FA synthesis in the mammary gland epithelial cells uses glucose, glycerol, and FA, as well as acetate and butyrate. Glucose can be used in 1 of 3 ways as it enters the mammary gland (Neville and Picciano, 1997). It may be used to generate acetyl-coenzyme A (CoA) through pyruvate and citrate synthesis, which will then be converted to malonyl-CoA and used as a carbon source for FA synthesis. Glucose may be converted to ribulose-5-phosphate through the pentose phosphate cycle to produce NADPH, a reducing equivalent. Glucose can also be used to generate glyceraldehyde-3-phosphate from the glycolytic chain which is then converted to glycerol-3-phosphate and used for triacylglycerol synthesis. Acetate and butyrate (converted to β-hydroxybutyrate by the ruminal epithelium) from ruminal fermentation serve as the main carbon sources. Acetate is used as the starting component to form acetyl-CoA. Acetyl-CoA carboxylase

catalyzes the reaction to convert acetate to malonyl-CoA. The conversion of acetate to malonyl-CoA is the rate-limiting step in the reaction (Neville and Picciano, 1997). The FA synthase enzyme condenses and adds carbon units to the chain (including butyryl Co-A), 2 at a time up to a length of C16:0. The addition of units is terminated by the enzyme thioesterase I (Barber et al., 1997).

Initial components of long-chain FA are absorbed from the blood stream as preformed lipids in the form of triglyceride-rich lipoproteins (chylomicra or very-low density lipoproteins) or NEFA (Bauman and Griinari, 2003). Lipoprotein lipase is active in bovine mammary tissue and is a key component in triglyceride uptake from the blood stream. Due to the activity of lipoprotein lipase, uptake of triglycerides is strongly related to plasma concentrations (Chilliard et al., 2000). Preformed FA absorbed from the blood stream are usually products of dietary consumption or microbial fermentation. High plasma NEFA concentrations correlate to higher NEFA uptake by the mammary gland. When a cow is in positive energy balance, less than 10% of milk FA is derived from NEFA, however, the proportion shifts when the cow enters negative energy balance (Bauman and Griinari, 2003). Bauman et al. (1988) treated cows in early lactation with recombinant bovine somatotropin, which causes shifts in nutrient partitioning, and observed an increase in milk energy yield and a 41% increase in milk fat yield. More than 90% of the increase in fat yield was associated with an increase in long-chain FA that are commonly derived from plasma NEFA and body fat reserves.

Preformed FA enter the mammary alveoli via diffusion or a saturatable membrane transport system (Neville and Picciano, 1997). Once the FA have entered the epithelial cells, acyl-CoA acts as a catalyst to begin the formation of triglycerides. Fatty acids from

de novo synthesis and those absorbed as pre-formed FA are esterified in the endoplasmic reticulum of mammary epithelial cells to form triglycerides. The FA are activated to their acyl-CoA esters and transacylases incorporate the FA onto a glycerol-3-phosphate (Neville and Picciano, 1997) to form a triglyceride.

A high delta-9 desaturase activity found in differentiated mammary epithelial cells is responsible for the conversion of saturated FA to monounsaturated FA (MUFA). Approximately 40% of mammary-absorbed stearic acid (C18:0) is desaturated to oleic acid (*cis*-9 C18:1) by delta-9 desaturase (Chilliard et al., 2000). The main isomer of CLA in ruminant milk is c*is*-9, *trans*-11 C18:2. Vaccenic acid (*trans*-11 C18:1) is formed in the rumen and absorbed into the blood stream through the intestine. After it has been absorbed from the blood by the mammary tissue, it is desaturated by delta-9 desaturase to form rumenic acid or *cis*-9, *trans*-11 C18:2 (Chilliard et al., 2000).

Milk fatty acid composition on pasture

The bovine milk FA profile is most commonly modified via a dietary route. One such method of modification is including pasture as a main component in the diet. Significant changes in milk FA composition when cows grazed pasture was observed by Jahreis et al. (1997), Kelly et al. (1998), Dhiman et al. (1999), White et al. (2001), Agenäs et al. (2002), Loor et al. (2003), and Ferlay et al. (2008) .

Milk samples were collected once a month for a year from the bulk tanks of 3 farms, each with different management practices (Jahreis et al., 1997). The first farm was a conventional operation with confinement feeding of corn silage the entire year. The second farm used conventional pasture without supplementation during the grazing

season and fed corn and grass silage in confinement during the other seasons. The third farm followed organic farming practices and grazed during the summer without supplementation and fed corn, grass, and alfalfa silage that had been produced in accordance with organic guidelines during the remainder of the year. Significant differences in milk FA composition were observed among all 3 production systems. Milk sample results for the year indicated that *trans*-11 C18:1 and CLA were significantly higher in bulk milk samples of both the conventional pasture system and the organic pasture system compared with the conventional farm, particularly during the grazing season (May to September).

Kelly et al. (1998) used 16 Holstein cows; 8 remained on a TMR diet and 8 were transitioned to a pasture-only diet. At the start of the study (May 12th), both groups received the same TMR, the pasture-only treatment group was transitioned from confinement feeding to grazing over the following 2 wk and remained on a pasture-only diet until the completion of the study on June 9th. A decrease in *de novo* FA synthesis was observed in cows on the pasture-only diet. Cows receiving TMR produced milk with 17.6% short- and medium-chain FA (of total milk FA), while cows consuming pasture produced milk with 12.8% short- and medium-chain FA (of total milk FA). Significant increases in oleic (+ 8.2%) and linolenic acid (+ 0.7%) and CLA (+ 0.63%) as a percent of total milk FA were observed in the final period for cows that grazed compared to consuming TMR.

Milk FA concentrations are related to the amount of pasture consumed (Dhiman et al., 1999). Holstein cows were assigned to 1 of 3 treatment groups and consumed 1/3 pasture, 2/3 pasture, or all pasture. Cows not on an all-pasture diet also received a

supplement of coarsely ground high moisture ear corn, alfalfa hay, roasted cracked soybeans, and soybean meal. Although concentrations of C10:0 to C16:0 did not differ between diets, a higher concentration of C16:1 was detected in the milk of cows on the pasture-only diet. Stearic acid was higher in the milk fat of cows receiving supplement, but oleic acid was not altered by diet. A linear increase in CLA concentration was observed as pasture increased from 1/3 to 2/3 to 100% of the diet resulting in 8.9, 14.3, and 22.1 g CLA/100 g FA, respectively. An increase in linolenic acid was also observed as pasture increased in the diet.

A TMR and grazing system was used to compare milk FA of Holsteins and Jerseys (White et al., 2001). The breeds were managed together while the treatment groups were managed separately. The TMR diet was composed of corn silage, alfalfa silage, ground corn, soybean meal, whole cottonseed, vitamins, and minerals. The cows on pasture grazed crabgrass and received a supplement mix with ground corn, soybean meal, whole cottonseed, vitamins, and minerals. Both breeds on the TMR treatment had a higher milk fat concentration than the breeds grazing pasture. However, the pasture cows produced significantly higher concentrations of CLA than the cows receiving TMR. Specifically, Jerseys consuming pasture produced milk with a CLA content of 0.59% of total FA compared to 0.32% of total FA for Jerseys consuming TMR. A similar increase in CLA was observed in Holsteins consuming pasture vs. Holsteins consuming TMR, 0.72 vs 0.41% of total FA, respectively. A significant increase in C10:0, C12:0, C14:0, C14:1, C16:1, and linolenic acid was noted in milk from the grazing cows compared to those receiving TMR.

Agenäs et al. (2002) noted significant increases in C18 FA 8 days after pasture turnout. Cows were assigned to either a concentrate supplement with high or low fat levels that were adjusted using soybean oil. Further, the cows were separated into 2 distinct populations: high fat indexed and low fat indexed. The Swedish Red cows used in the study were selected from a genetic program in which high producing cows were selected for high or low milk fat percentage, but the same amount of energy in the milk (Agenäs et al., 2003). The short-term study lasted 29 d with cows evaluated on d 1 as the indoor system, d 8 as the pasture transition period, and d 29 as the pasture period. During the transition period (d 8), when adaptation to pasture was occurring, a decrease in *de novo* FA was observed, while at the same time an increase in long chain FA was shown. Pasture is high in unsaturated FA (NRC, 2001) and the high level of unsaturated FA in combination with a higher rate of ruminal passage often observed with pasture-based diets (Kelly et al., 1998; Bargo et al., 2003) may have led to incomplete saturation of the FA and could have contributed to the shift in milk fat composition. Stearic acid was not altered by turnout to pasture. However, an increase in linoleic and linolenic acid was observed during the adaptation period (d 1 to d 8). After turnout to pasture, the sum of all *cis*-C18:1 isomers increased and remained elevated through the remainder of the study. In addition, the sum of all *trans*-C18:1 isomers and CLA were higher on d 8 and d 29 than on d 1. Cows in the low fat indexed population receiving the high fat concentrate demonstrated the highest *trans*-C18:1 and CLA contents. The authors suggested the difference was due to cows in the low fat indexed population having a lower ability for *de novo* FA synthesis. In a previous study (Murphy et al., 2000), low fat indexed cows

tended to have higher ruminal fluid passage rates compared to high fat indexed cows, which could increase the amount of incompletely saturated FA passing from the rumen.

Milk FA composition may be affected by time of day that grazing takes place and is significantly altered by 8 h of grazing (Loor et al., 2003). Three treatment groups (TMR only, TMR plus 8 h grazing following morning milking, or TMR plus 8 h grazing following afternoon milking) were established with 10 cows each. Although DMI differed between the 3 treatments, there was no difference detected in milk production, or percentage and yield of milk fat, protein, and lactose. Significant decreases in C14:0 and *trans*-10 C18:0 were observed when cows grazed for 8 h compared to a complete TMR diet. Vaccenic acid (*trans*-11 C18:1) was significantly increased in cows grazing 8 h and was significantly higher in the milk of cows grazing in the afternoon. Linoleic acid was significantly decreased in cows consuming pasture but the *trans*-11, *cis*-15 C18:2 isomer was significantly increased as was linolenic acid. Conjugated linoleic acid was also increased with 8 h of grazing and was significantly higher when cows grazed afternoon compared to morning. The authors suggested that increased DMI from pasture when cows grazed in the afternoon compared to the morning contributed to the difference, although pasture intake was not quantified. Cows grazing in the afternoon had a significantly lower TMR DMI than those grazing in the morning. However, milk yield did not differ between the 2 groups, suggesting that cows maintained production via increased pasture intake.

Soder et al. (2006) grazed 20 lactating cows on 4 different forage mixtures for 2 grazing seasons to evaluate the effects of increased forage species. The 4 forage mixtures were: 1) 2 species: orchardgrass and white clover, 2) 3 species: orchardgrass, white

clover, and chicory, 3) 6 species: orchardgrass, tall fescue, perennial ryegrass, birdsfoot trefoil, red clover, and chicory, 4) 9 species: all species included in the 6 species mix plus white clover, alfalfa, and Kentucky bluegrass. Forage mix did not affect DMI, milk production, or 4% FCM. The milk fat of cows consuming the forage mix with only 2 species produced significantly lower levels of linoleic acid and CLA than those consuming the mixes containing more species. The mixes containing more than two species also contained chicory, which was reported to have increased unsaturated FA concentration and may have contributed to the differences in milk FA. However, sesquiterpene lactones (Foster et al., 2002) found in chicory can cause a bitter taste and cows may have avoided grazing the mature leaves and bolting stem. The authors suggested higher legume intake, due to chicory avoidance in the treatment groups grazing more than 2 species, may have contributed to the difference in milk FA because legumes are higher in linoleic acid than cool-season grasses.

Ferlay et al. (2008) evaluated variability of pooled bulk tank milk samples 2 times during the winter and 3 times during the grazing season. A region located in south central France was separated into 10 areas and farms within each area (n = 10 to 36) contributed to the pooled milk sample on a basis of production percentage within that area. Significantly higher production of C10:0 to C14:0 was observed during the winter feeding season as compared to the grazing season. A linear decrease of C10:0 to C14:0 was observed when winter herds feeding a high percentage of preserved forages was compared to summer grazing herds feeding a low percentage of preserved forages. The sum of *trans*-C18:1 FA isomers (including *trans*-11 C18:1) were significantly increased in milk samples collected during the grazing season than those collected during the

winter. *Cis*-9, *trans*-11 CLA was significantly increased during the grazing season compared to the winter season.

Pasture consumption has marked effects on milk FA composition, increasing MUFA and PUFA concentrations as well as CLA and n-3 FA. Improvements in the milk FA profile holds potential benefits for human health and may encourage more extensive use of pasture in modern dairy operations.

Milk fatty acid composition with flaxseed supplementation

Significant differences in milk FA composition were detected when a control diet was supplemented or not with ground, raw flaxseed, micronized flaxseed, or extruded flaxseed (Gonthier et al., 2005). Micronized flaxseed is a method of processing flaxseed to produce smaller particle size and may reduce ruminal degradability while increasing post-ruminal digestibility. Extrusion of flaxseed is a heat treatment that may help to protect the product from ruminal degradation by the microbes. A decrease in short- and medium-chain FA along with an increase in long-chain FA was observed in cows receiving flaxseed supplementation. An average increase of 193 and 51% was noted for linolenic acid and CLA, respectively, when flaxseed was fed. In addition, daily yield of CLA was increased by an average of 4 g/d due to all flaxseed treatments.

Bell et al. (2006) supplemented flaxseed oil at a rate of 6% of diet DM in conjunction with Vitamin E at 150 IU/kg of DM to a control diet of barley silage, alfalfa silage, alfalfa hay, ground corn, barley, soybean meal, and corn gluten meal. Cows receiving the flaxseed/vitamin E supplemented diet had significantly higher levels of linoleic acid, linolenic acid, and CLA in the milk fat than cows on the control diet. In

addition, saturated FA were notably decreased while unsaturated FA were increased in the milk fat of cows consuming flaxseed oil compared to the unsupplemented cows.

A quadratic increase in milk *cis*-9, *trans*-11 CLA content was reported by Flowers et al. (2008) when grazing cows were supplemented with increasing levels of flaxseed oil. Flaxseed oil was added to a corn-based supplement to yield 4 treatment groups: 1) 0 g/d flaxseed oil, 2) 170 g/d flaxseed oil, 3) 340 g/d flaxseed oil, and 4) 510 g/d flaxseed oil. Additionally, n-3 FA and linolenic acid concentrations increased as flaxseed oil in the diet increased.

Rego et al. (2009) supplemented cows grazing pasture for 20 h per d with 5 kg of corn concentrate, 4.5 kg concentrate plus 0.5 kg canola oil, 4.5 kg concentrate plus 0.5 kg sunflower oil, or 4.5 kg concentrate plus 0.5 kg flaxseed oil. Oil supplementation decreased short- and medium-chain milk FA. However, flaxseed oil supplementation also decreased linoleic and linolenic acid. A 19% increase in *cis*-monounsaturated FA was noted with the flaxseed oil diet.

Petit and Côrtes (2010) evaluated milk FA composition when a TMR diet was supplemented with calcium salts of palm oil (control diet), 7.2% DM whole flaxseed, 7.2% DM GFLAX, or 3.6% DM whole flaxseed plus 3.6% DM GFLAX. The cows receiving GFLAX and the combination treatment of whole flaxseed plus GFLAX produced higher proportions of linolenic acid when compared with the whole flaxseed treatment. Cows on the GFLAX treatment produced higher proportions of total n-3 FA compared to the cows on the whole-ground flaxseed diet, but no other effects of processing were observed. Cows consuming the control diet produced milk with higher proportions of palmitic acid and *cis*-7 C16:1 and lower proportions of stearic and oleic

acid than cows on flaxseed treatments. Conjugated linoleic acid production did not differ among the 4 treatments.

Long-term supplementation with extruded flaxseed was evaluated by Lerch et al. (2012b) during a confinement as well as a grazing period for 2 consecutive lactations. During the confinement period of the first year, supplementation with extruded flaxseed decreased milk saturated FA, and increased total *cis*-C18:1 and *trans*-C18:1 concentrations compared with the control in which the basal diet was supplemented with a pelleted wheat and solvent-extracted rapeseed meal. During the grazing period, an increase of 8.3 g PUFA/100 g FA was observed when the control diet was compared to the indoor period. Extruded flaxseed supplementation during the grazing period increased milk total PUFA by 1.6 g PUFA/100 g FA compared to the indoor period. In the second year of the study, extruded flaxseed supplementation led to greater increases in total *trans*-C18:1, PUFA, and linolenic acid during the confinement period when compared to the first year. When the second grazing period was compared to the first grazing period, cows receiving extruded flaxseed demonstrated greater increases in linoleic acid and n-3 PUFA. Changes of milk FA due to supplementation were more notable during confinement periods than grazing periods. An unexpected observation was reported by Lerch et al. (2012c) during both grazing periods. Cows receiving extruded flaxseed produced significantly lower concentrations of CLA compared with the cows on the control diet, which was attributed to a higher starch content in the control diet from the pelleted concentrate supplementation than the extruded flaxseed diet.

Forage to concentrate ratio, in addition to flaxseed supplementation, impacts milk FA composition (Neveu et al., 2013). Four diets were fed: 1) high forage (60% of DM)

without flaxseed, 2) high forage (60% of DM) with extruded flaxseed (9% of DM), 3) low forage (40% of DM) without flaxseed, and 4) low forage (40% of DM) with extruded flaxseed (9% of DM). Flaxseed supplementation did not affect short-chain FA C4:0 to C14:0, however, significantly higher concentrations of C4:0 to C8:0 and significantly lower concentrations of C12:0 and C14:0 were detected when cows were fed high forage diets. When cows were fed high forage diets, an increase in C16:0 was noted and C16:0 was decreased when extruded flaxseed was fed. High forage diets containing flaxseed, high forage diets, and diets containing flaxseed all resulted in significant increases in stearic acid. A 100 and 54% increase in linolenic acid and CLA, respectively, was reported when flaxseed was fed. Conjugated linoleic acid was increased in high forage diets, but linolenic acid did not increase. The results of this study indicate that supplementing high forage diets with flaxseed may be beneficial for increasing FA with potential human health benefits.

Flaxseed contains high levels of unsaturated FA and feeding flaxseed in various forms has been shown to alter milk FA composition. The degree to which milk FA is altered may vary depending on basal diet, flaxseed form, and intake level.

Premiums for nutritionally enriched milk fat

In a relatively new program, the Organic Valley Family of Farms cooperative currently markets and sells a product labeled “100% grassmilk” in which the cows producing the milk consume only fresh pasture herbage or dried forages such as hay and no grain is included in the diet (Organic Valley, 2014). This milk is marketed as providing “naturally occurring omega-3 and CLA” other marketed products such as the

"pasture butter" and "grass milk cheese" also indicate they contain higher levels of n-3 and CLA. New England farmers who are a part of the grass milk program at Organic Valley cooperative are currently (December 2014) receiving a premium of $5/cwt higher ($4 as a premium, $1 credit dedicated to soil amendments) than farmers utilizing conventional organic practices, $36.05 and 30.80, respectively (CROPP Cooperative, 2014).

Organic Valley does currently offer a premium to poultry producers for n-3 enriched eggs (+ $0.14/dozen) compared to conventional organic eggs (CROPP Cooperative, 2014). Although the dairy products being marketed advertise enriched n-3 and CLA content, there is not currently a premium in place for achieving specific levels of n-3 or CLA. Organic Valley indicated a premium for enriched n-3 and CLA concentrations has been considered in the past and may develop in the future because farmers have expressed interest in a program. (H. Chappell, Organic Valley, La Farge, WI; personal communication).

Conclusions

Evaluating the impacts of flaxseed supplementation during the grazing season is an important area for future research. Supplementation may promote increased production of FA that are beneficial to human health and may minimize season variations due to shifting of pasture species throughout the grazing season. If flaxseed supplementation is economically viable, it may contribute to an emerging specialty market featuring health-enriched dairy foods.

CHAPTER II. EFFECTS OF GROUND FLAXSEED SUPPLEMENTATION ON PRODUCTION, MILK FATTY ACID COMPOSITION, AND ENTERIC METHANE PRODUCTION DURING THE GRAZING SEASON

MATERIALS AND METHODS

Experimental design and treatments

Twenty multiparous organically-certified lactating Jersey cows were assigned to 1 of 2 treatments using a randomized complete block design. One cow on the flaxseed treatment died due to reasons unrelated to experimental diets during the second month of the study and was replaced by a multiparous cow of similar days in milk (DIM). Organic flaxseed, ground via a cold milling process, was obtained from AgMotion Inc., (Minneapolis, MN). Dietary treatments were 0 or 10% of GFLAX in diet DM. All cows were fed the same basal diet (Table 1). Cows on the control treatment (0% GFLAX) were fed a ground corn-soybean meal mixture at 10% of diet DM (Table 2). Dietary treatments were weighed separately, top dressed, and manually mixed into the individual TMR for each cow at each feeding. Diets were formulated to provide similar net energy of lactation (NE_L) concentrations and 60% of DMI from TMR with the remaining 40% from pasture.

At the start of the trial, cows averaged 111 ± 49 DIM. The replacement cow assigned to the study during the second month was 128 DIM. The study extended from

June 8 to September 27, 2013. The study was conducted over 4, 28-d periods with the last 7 d of each period used for data and sample collection. Total mixed ration intake was measured daily, with TMR DM determined once weekly during the first 3 wk and daily during the final wk of each period. Cows were fed in individual Calan doors (American Calan, Northwood, NH).

Management of cows

All procedures related to animal care were conducted with the approval of the University of New Hampshire Institutional Animal Care and Use Committee (Appendix A). Cows were housed in a bedded pack barn with an open lot and covered feeding area. Cows were milked and fed twice daily at 0630 and 1730 h. Cows were milked in a 4-stall step-up parlor with headlocks (Agromatic; Fond DuLac, WI), automatic take-offs, and milk meters (Westfalia Surge; GEA Farm Technologies Inc., Naperville, IL). Milk weights were recorded every day (DairyPlan C21, Version 5.2; GEA Farm Technologies Inc., Naperville, IL). Cows were weighed (Northeast Scale Co., Inc., Hooksett, NH) on 3 consecutive days at the beginning of the experiment and during the last week of each period. Three independent scorers assigned BCS at the beginning of the experiment and in the last week of each period. The mean BW and BCS at the start of the experiment was 408 ± 44 kg and 2.93 ± 0.31, respectively.

The basal diet was fed as a TMR and was prepared by weighing each ingredient and mixing in a vertical mixer (V-Mix 400; ValMetal, Tomah, WI) using a Maxxum series tractor (MX 135; Case IH, Sturtevant, WI). The TMR was prepared fresh in the evening, dispensed onto concrete, and weighed into barrels using a portable digital scale

(Pelouze 4010; Rubbermaid, Saratoga Springs, NY). The remaining TMR was fed during feeding the following morning. Orts were weighed and collected daily at 1500 h. Cows were allowed approximately 60 to 90 min to consume the TMR in both daily feedings and were then moved to pasture. Pasture was strip-grazed using a temporary fencing system (polywire and step-in fence posts) and cows had access to water throughout the experiment.

Chromium oxide was incorporated into a pelleted grain mash (Morrison's Custom Feeds, Barnet, VT) with similar composition to that of the basal concentrate mix (Table 2), and was used as an external marker to estimate pasture DMI. During the last 10 d of each period, a portion of the grain in the basal diet was substituted for 833 g (DM basis) of the Cr_2O_3 pellets, which were offered twice daily before each feeding in rubber pans and placed into the Calan doors to ensure complete consumption of all pellets. Target intake was 10 g of Cr_2O_3/d.

Pasture management and sampling

During the first 3 wk of each period, cows used in the study (n = 20) and the remaining lactating animals (n = 30) of the herd grazed in the same group. During the sample collection weeks, cows assigned to the study grazed separately. In the first 3 wk of each period, pasture herbage samples were collected once weekly and pasture area was recorded. Herbage samples were collected each time cows were moved to a new strip of fresh herbage during the sample collection weeks. Cows were moved to a new strip once daily. Pasture sections were mapped with a GPS device (Garmin Ltd., Olathe, KS) and total area (m^2) was recorded. Pre-grazing and post-grazing herbage mass (kg DM/ha) was

measured by cutting 10 quadrats (0.25 m^2/quadrat) of herbage to ground level using hand shears. Herbage quality was evaluated by hand-plucking 10 quadrats (0.25 m^2/quadrat) of herbage to a level approximating the post-grazing herbage mass (Kolver and Muller, 1998). Quadrats were thrown in a "zig-zag" pattern in each pasture section for both herbage mass and quality. Biomass and quality samples were collected adjacent to one another in areas containing similar forages to maintain consistency. If the locations of the quadrat throw contained manure, it was thrown again. After collection, 500 g (wet weight) of herbage was separated from the pre-grazing herbage mass sample by hand-mixing and quartering the total sample until only 500 g remained. The remaining 500 g was sorted into 1 of 4 categories: 1) grass, 2) legume, 3) weed, or 4) dead and used to estimate herbage composition. The weights of sorted herbage composition samples were included in total calculations of pre-grazing herbage biomass. All herbage samples were dried at 55°C in a forced air oven (Sheldon Manufacturing, Inc., Cornelius, OR; BINDER Inc., Bohemia, NY; VWR Scientific, Bridgeport, NJ).

Herbage biomass was calculated by multiplying total area harvested in the quadrats (2.5 m^2) by the total area of the paddock. Total pre-grazing herbage biomass was used to calculate the pasture allowance on a per cow basis.

Feed sampling and analysis

Concentrates were sampled once during each of the 4 sample collection weeks for nutrient analysis. Each bale of baleage was sampled via core-sampling with an electric drill (Hilti Inc., Tulsa, OK) and a 45-cm stainless steel forage sampler barrel (Nasco, Fort Atkinson, WI). Total mixed ration and orts were sampled daily during each sample

collection week. All feed samples were dried for 48 h in a forced hot air oven at 55°C (Sheldon Manufacturing, Inc., Cornelius, OR; BINDER Inc., Bohemia, NY; VWR International, Bridgeport, NJ). The dried baleage, concentrates, TMR, orts, and pasture samples were ground to pass through a 1-mm screen using a Wiley Mill (Thomas Scientific, Swedesboro, NJ). Composites were made of each dried feed across each sample collection week. Composites were analyzed for CP, ADF, NDF, crude fat, ash, acid insoluble ash, Ca, P, Mg, K, Fe, Zn, Cu, Mn, S, and Cr (Analab, Agri-King, Inc., Fulton, IL). Crude protein was determined using the combustion method (AOAC, 990.03, 1990), ADF was determined using acid detergent solution (AOAC 973.18, 1990), NDF was determined using amylase-treated neutral detergent solution (AOAC 2002.04, 1990), and crude fat was determined using ether extraction (AOAC 920.39, 1990). Ash was determined using the combustion method (AOAC 942.05, 1990), AIA was determined using techniques described in Van Keulen et al. (1977), and minerals were determined using the inductively coupled plasma spectroscopic method (AOAC 985.01, 1990) with the exception of sulfur determined by magnesium nitrate methodology (AOAC 923.01, 1990) and chromium determined by atomic absorption (Williams et al., 1962; Binnerts et al., 1968). Indigestible acid detergent fiber (iADF) was also measured in composite feed samples. Composited feeds (0.45 – 0.55 g/bag) that had been dried and ground as previously described, were incubated in triplicate in the rumen of a cannulated Holstein cow consuming a TMR diet (40.4% corn silage, 11.4% soybean meal, 11.2% grass-legume haylage, 9.86% ground corn grain). Bags (F57, ANKOM, Macedon, NY) were incubated in the rumen for 12 d using the filter bag technique. Following incubation, bags were removed, washed, and analyzed for ADF using ANKOM Method 8 (ANKOM,

Macedon, NY). Composited feed samples were evaluated for FA content (Dr. Kevin Harvatine Laboratory; The Pennsylvania State University, University Park, PA) following the procedure of Rico et al. (2014). In vitro dry matter digestibility (IVDMD) of composited feed samples was measured by Cumberland Valley Analytical Services (Hagerstown, MD) using the methodology described by Tilley and Terry (1963). Grains and forages were assessed for 48 and 72 h IVDMD, respectively.

Rumen sampling and analysis

Ruminal samples were collected from 10 cows after the a.m. milking for 3 consecutive days during each sample collection week. During sample collection, the cows were restrained in a head chute (Zimmerman, PBZ LLC., Lititz, PA) with belly straps to minimize movement. Samples were collected using an oral lavage tube passed through the esophagus and into the rumen. Ruminal fluid was extracted by applying a manual vacuum and the initial portion of the sample was discarded to minimize saliva contamination. The sample was strained through 4 layers of cheesecloth and pH (VWR International, Bridgeport, NJ) was recorded immediately. A 40-mL aliquot of strained ruminal fluid was added to 2.4 mL of 6 *N* HCl, stored at -20°C, and retained for analysis of ammonia. Analysis of ruminal ammonia was performed by adding 1 mL of ionic strength adjuster (Ammonia pH adjusting ISA; Orion 951211, Thermo Fisher Scientific, Chelmsford, MA) to 10 mL of ruminal fluid and measuring the released ammonia with a benchtop pH/ISE meter (Orion Star A214; Thermo Fisher Scientific, Chelmsford, MA). A 40-mL aliquot of strained ruminal fluid was added to 0.18 mL of 50% H_2SO_4, stored at -20°C, and retained for VFA analysis. Analysis of ruminal fluid for VFA was done at

West Virginia University (Morgantown, WV) using gas chromatography (Anonymous, 1975). The gas chromatograph was a Varian model 3300 with a flame ionization detector (Varian, Inc., Palo Alto, CA). The column was a 2-m × 2-mm glass column packed with 10% stationary phase 1200/1 H_3PO_4 on 80/100 Chromosorb W-AW media (Supelco Inc., Bellefonte, PA).

Blood sampling and analysis

Blood was sampled by venipuncture of the coccygeal vein or artery before the a.m. and p.m. milking for the first two consecutive days (d 1 and 2) of each sampling week. Blood was collected in 10 mL evacuated-glycerin coated red stopper tubes (Monoject; Covidien, Mansfield, MA) with a 20-gauge blood collection needle (Monoject; Covidien, Mansfield, MA). Blood samples clotted at room temperature for approximately 1 to 2 h and then were centrifuged for 20 min at 1,200 × *g* at room temperature. Serum was collected with a.m. and p.m. samples pooled in equal amounts by day. Samples for analysis of serum cortisol were stored at -80°C while remaining samples were frozen at -20°C until analysis. The BioVendor (BioVendor LLC, Asheville, NC) cortisol ELISA kit was used to evaluate serum cortisol. Analysis of serum NEFA was performed using the Wako HR Series NEFA-HR(2) kit (Wako Chemicals USA, Inc., Richmond, VA) modified for bovine use. A chromate microplate reader (Awareness Technology, Inc., Palm City, FL) was used to read absorbance of both NEFA (550 nm) and cortisol (450 nm) assays. Serum urea was determined via colorimetric analysis using a UV/visible spectrophotometer (Beckman Coulter Inc., Brea, CA) set at a wavelength of 540 nm.

Urine and fecal sampling and analysis

Urine samples were collected twice daily before the a.m. and p.m. milking for 3 consecutive days (d 3, 4, and 5 of the sampling week). Samples were composited to create 1 sample per cow/period by adding 8 mL of urine and acidifying with 400 µL of 6 *N* HCl per each time point. Urine samples were stored at -20°C until later analysis for N, ammonia, creatinine, urea, allantoin, and uric acid. Urinary N concentration was assessed by Dairy One Cooperative Inc. (Ithaca, NY) using a Leco TruMac N Macro Determinator (Leco Corporation, St. Joseph, MI) using the combustion method (AOAC 990.03, 1990). A benchtop pH/ISE meter (Orion Star A214; Thermo Fisher Scientific, Chelmsford, MA) was used to measure urine ammonia following the same methodology used for ruminal samples. Creatinine concentration was measured using the Cayman Chemical Company (Ann Arbor, MI) creatinine colorimetric kit on a Chromate microplate reader (Awareness Technology, Inc., Palm City, FL) at 492 nm. Urinary urea was determined via colorimetric analysis. Allantoin concentration was determined through a modified procedure (Chen, 1989). Measurement of uric acid was performed using the Stanbio Uric Acid LiquiColor kit (Procedure no. 1045; Stanbio Laboratory, Boerne, TX). Urinary urea, allantoin, and uric acid were read at wavelengths of 540, 522, and 520 nm, respectively, on a UV/visible spectrophotometer (Beckman Coulter Inc., Brea, CA).

Fecal samples were collected twice daily before the a.m. and p.m. milking during the last 5 d of each sample collection week. Samples were pooled by cow and dried in a forced air oven at 55°C (Sheldon Manufacturing, Inc., Cornelius, OR). Samples were ground to pass through a 1-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ). Samples were analyzed for CP, ADF, NDF, ash, acid insoluble ash,

and Cr by Analab (Agri-King, Inc., Fulton, IL) as previously reported. Fecal samples were also analyzed for iADF as previously reported.

Fecal chromium concentration was used to calculate fecal output using the equation: fecal output = (g per day of Cr) ÷ (g of Cr/g of fecal DM) according to Kolver and Muller (1998). Pasture DMI was estimated using the following equation (Bargo et al., 2002): pasture DMI = [(g Cr/d) ÷ (g Cr/g of fecal DM) – concentrate DMI × (1 – IVDMD of concentrate) – TMR DMI × (1 – IVDMD of TMR)] ÷ (1 – IVDMD of pasture).

Milk sampling and analysis

Milk samples were collected during both a.m. and p.m. milking times for 2 consecutive days (d 1 and 2) during each sample collection week. Samples were composited by day based on individual production, preserved with a 2-bromo-2-nitropropan-1,3 diol tablet (D&F Control Systems, Inc., Norwood, MA) and analyzed for fat, protein, lactose, somatic cell count, and MUN by Dairy One Cooperative (Ithaca, NY). A composite of all 4 time points, based on individual milk yield by day, was retained for analysis of milk FA and stored at -80°C. Milk FA were analyzed in the same laboratory used for feed FA following a modified procedure (Rico and Harvatine, 2013). The oven program was modified to an initial temperature of 80°C and increased 2°C per minute to 190°C and held for 10 min. The temperature was then increased 8°C per minute to 215°C and held for 25 min.

Energy corrected milk yield was estimated according to Orth (1992) and 4% FCM was estimated according to Gaines and Davidson (1923). Feed efficiency was determined by using the following ratios: milk yield/DMI, ECM/DMI, and FCM/DMI.

Methane and carbon dioxide measurements

Expired CH_4 and CO_2 were measured using a portable automated gas quantification system (GreenFeed; C-lock, Rapid City, SD). The gas quantification system was mounted on a trailer and moved to each new paddock location with the cows. The cows were identified with radio frequency identification tags to permit access to the unit; 18 cows used the system and all recorded measurements for at least one month. The unit was set for 4 daily visits with alfalfa pellets (Table 5) being dispensed a maximum of 5 times per visit (50 g/dispense).

Collection of covariate period samples

One week prior to the start of the study, selected covariate samples were collected. These samples included milk, blood, urine, and feces. Dry matter intake of TMR was also recorded. The covariate samples were analyzed according to the same procedures detailed above and were used in statistical analysis of most of the data sets.

Calculations

Nitrogen excretion was calculated as total manure N excretion as the sum of fecal N and urinary N. Creatinine concentration was calculated using one mean daily creatinine excretion rate (29 mg/kg of BW per d) according to Valadares et al. (1999): BW × 29 ÷

creatinine concentration (mg/L). Total purine derivative (PD) excretion was calculated according to Chizzotti et al. (2008) as: PD:creatinine (mmol/L) × creatinine excretion (mmol/d), where PD is the sum of the concentration of allantoin and uric acid (mmol/L). Temperature-humidity index (THI) was calculated according to Dikmen and Hansen (2009): $(1.8 \times td) - [(0.55 - 0.0055 \times RH)(1.8 \times td - 26.80)]$, where td is the dry bulb temperature in °C and RH is the relative percent humidity.

Income over feed cost analysis

Income over feed cost was calculated by using the actual milk price to calculate milk income and subtracting the feed cost to determine IOFC per cow/d. Actual data obtained from farm records for the time period of the experiment was used to calculate IOFC. Actual data obtained from farm prices included: milk price receipts, grain mix costs, ground flaxseed costs, and molasses costs (Table 12). The price of home-grown forages (baleage and pasture) were obtained from the Penn State Feed Price List (Ishler, 2013).

Statistical models

Covariate data and data collected in June from the cow that died in July remained in the model and the last 3 months of the experiment were entered as missing values. Data collected in July was used to establish covariate values for the replacement cow and data collected in the last 2 months of the experiment were used as experimental results. Data for milk components and blood parameters were analyzed using the REPEATED

procedure of SAS 9.3 (2010) according to the following model to determine if day was significant;

$$Y_{ijklp} = \mu + B_i + T_j + M_k + D_l + MD_{kl} + KC_{ijklp} + E_{ijklp}$$

Where:

Y_{ijklp} = is the dependent variable

μ = overall mean

B_i = is the fixed effect of the i^{th} block; i = 1…10

T_j = is the fixed effect of the j^{th} treatment; j = 1…2

M_k = is the fixed effect of the k^{th} month; k = 1…4

D_l = is the fixed effect of the l^{th} day; l = 1…2

MD_{kl} = is the fixed effect of the interaction between the k^{th} month and the l^{th} day

K = is the regression coefficient of the covariate C

C_{ijklp} = is the value of the covariate variable for the p^{th} cow within the i^{th} block within the l^{th} day of the j^{th} treatment; p = 1…20

E_{ijklp} = is the random residual ~$N(0, \sigma^2_e)$

In this model, the random effect of cow was used as the error term for the effect of treatment. Degrees of freedom were calculated using the Kenward-Roger option of MIXED procedure (SAS, 2010). Significant treatment levels were noted at $P \leq 0.05$ while $0.05 < P \leq 0.10$ was declared as a trend.

Data were analyzed for effect of block, which was not significant and removed from the final model. Day was not significant for any milk or blood parameters and the means for the 2 d were calculated. Milk, blood, urine, ruminal VFA, and DMI data were

analyzed using the PROC MIXED procedure of SAS 9.3 (2010) according to the following model:

$$Y_{ijk} = \mu + L_i + M_j + L \times M_{ij} + KC_{ijk} + E_{ijk}$$

Where:

Y_{ijk} = is the dependent variable

μ = overall mean

L_i = is the fixed effect of the i^{th} treatment; i = 1…2

M_j = is the fixed effect of the j^{th} month; j = 1…4

$L \times M_{ij}$ = is the interaction between the i^{th} treatment and the j^{th} month

K = is the regression coefficient of the covariate C

C_{ijk} = is the value of the covariate variable for the l^{th} cow within the k^{th} treatment; l = 1…20

E_{ijk} = is the random residual ~N(0, σ^2_e)

In this model the random effect of cow nested within treatment was used as the error term for the effect of treatment. Degrees of freedom were calculated using the Kenward-Roger option of MIXED procedure (SAS, 2010). Least square means were determined for treatments. The pdiff option of LSM was used to determine differences between treatments and months. Significant levels were noted at $P \leq 0.05$ while $0.05 < P \leq 0.10$ was declared a trend.

The PROC MIXED procedure of SAS 9.3 (2010) was selected because the data set contains fixed- and random-effects parameters. In addition, the PROC MIXED procedure allows for evaluation of several covariance structures (ie.; compound symmetry, unstructured, autoregressive and etc.) to determine the one best fitted to the

data as well as inclusion of blocks and the ability to handle unbalanced data as seen in this data set where several values were missing.

RESULTS

Weather data

Weather data was collected from the Portsmouth International Airport, Portsmouth, NH. The average daily temperature throughout the study was 20.0°C, and the average low and average high temperatures were 14.9°C and 25.4°C, respectively. The average temperatures for June, July, August, and September were 20.1, 22.7, 20.5, and 16.8°C, respectively. The average THI throughout the study was 66.9 and the average THI for June, July, August, and September was 67.0, 71.2, 67.2, and 62.0, respectively. The average daily precipitation throughout the study was 4 mm and the total precipitation was 456 mm.

Nutrient composition

The ingredient composition of the diet is detailed in Table 1 and the ingredient composition of the grain mixes is shown in Table 2. The chemical composition of the feeds is listed in Table 3 and the chemical composition of the pasture as well as pasture management data are listed in Table 4. The NRC (2001) evaluation of the diet is presented in Table 5. The NE_L supplied by the 0 and 10% GFLAX diets was 26.4 and 28.4 Mcal/d, respectively (Table 5). The FA composition of the feeds is listed in Table 6.

Production and nutrient digestibility

Total dry matter intake, milk yield, and milk component yields and concentrations were not affected by GFLAX supplementation (Table 7). Similarly, ECM and 4% FCM were not affected by dietary treatment (Table 7). A trend for increased milk urea N

(MUN) (P = 0.10) was noted for cows on the 0% GFLAX diet. Feed efficiencies did not differ between treatments (Table 7). There were no differences in somatic cell score (SCS), BW, BCS, plasma NEFA, or serum cortisol in the 2 treatments (Table 7). Plasma urea N was higher (P = 0.01) in cows on the 10% GFLAX diet.

Differences among months were observed for total DMI, milk yield, and milk protein and lactose concentrations (Table 7). Differences among months were also reported for milk protein yield. In addition, MUN, feed efficiency, SCS, BW, BCS, plasma urea nitrogen (PUN), and plasma NEFA showed differences among months. The only variable with a significant treatment × month interaction was MUN.

Apparent total tract nutrient digestibility, calculated using chromium oxide and IVDMD are presented in Table 8. Total DMI was not different between the 2 treatment groups, however, a trend (P = 0.07) for higher pasture intake was observed in the 10% GFLAX diet. Dry matter digestibility was significantly lower (P = 0.01) in cows fed the 10% GFLAX diet. Organic matter (P = 0.01) and CP (P = 0.05) digestibility were significantly lower in the 0% GFLAX diet. A trend (P = 0.10) for higher NDF digestibility was observed with the 10% GFLAX treatment. All digestibility parameters (Table 8) varied due to effects of month and treatment × month interactions were observed for OM (P < 0.05) and ADF (P = 0.05) digestibility.

Apparent total tract nutrient digestibility, calculated using chromium oxide and AIA, are presented in Table 8. Total DMI did not differ between the 2 treatment groups, however, a trend (P = 0.07) for higher pasture intake was detected with the 10% GFLAX treatment. Dry matter and ADF digestibility were higher (P < 0.001) in the 0% GFLAX diet. Organic matter digestibility was lower (P < 0.001) on the 0% GFLAX treatment.

Differences due to month were noted for all factors (Table 8) and a treatment × month interaction was observed for OM digestibility.

Apparent total tract nutrient digestibility, calculated using chromium oxide and iADF are listed in Table 8. Total DMI was not different between treatment groups and a trend ($P = 0.07$) for higher pasture intake was observed in the 10% GFLAX diet. Organic matter ($P < 0.01$), NDF ($P = 0.05$), and CP ($P < 0.01$) digestibility were higher in the 10% GFLAX diet. A trend ($P = 0.07$) for higher DM digestibility was observed in the 0% GFLAX treatment group. Differences due to month were noted for all factors. Treatment × month interactions were observed for OM ($P < 0.05$) and CP ($P < 0.05$) digestibility.

Ruminal pH and volatile fatty acids

Ruminal pH and VFA results are presented in Table 9. No effects of diet on ruminal pH were detected. The ratios of acetic to propionic acid and acetic+butyric to propionic acid were both higher ($P < 0.01$) in the 0% GFLAX diet. Diet did not affect individual or total VFA concentrations as shown in Table 9. When expressed as a proportion of total VFA concentration, acetic acid showed a trend ($P = 0.06$) for increased proportion, and butyric acid was increased ($P = 0.02$) in the 0% GFLAX diet. The proportion of propionic acid was higher ($P < 0.01$) in the ruminal fluid of cows on the 10% GFLAX diet. Effects of month were observed for all ruminal parameters with the exception of acetic acid concentration which demonstrated a trend ($P = 0.07$) for monthly flucuations. No interactions of treatment × month were noted for ruminal pH or VFA concentrations or proportions.

Enteric methane production

Enteric CH_4 and CO_2 production were not affected by dietary treatment with GFLAX (Table 9). An effect of month was noted for both parameters, however, no interaction of treatment × month was detected.

Nitrogen intake and excretion

Nitrogen intake (Table 10) was higher ($P = 0.01$), 470 vs. 446 g/d, in 10% GFLAX diets compared to 0% GFLAX because of the higher CP found in GFLAX compared to corn/soybean control treatment. Urinary creatinine concentration demonstrated a trend ($P = 0.08$) for higher excretion in the 10% GFLAX diet. Excretion of purine derivatives was not affected by dietary inclusion of GFLAX (Table 10). Urea N excretion was higher ($P = 0.03$) for cows on the 10% GFLAX diet, however, no other N excretion parameters were affected by dietary treatment. Differences due to month were observed for N intake, urinary concentration and excretion of purine derivatives, urinary excretion of N, urea N, and NH_4. Increased N intake during July and September is correlated to higher legume content of pasture herbage which translated to higher herbage CP content. Higher N intake due to increased herbage CP is supported by increased N excretion parameters during July and September. No treatment × month interactions were observed for any of the parameters.

Milk fatty acid concentration

Milk FA proportions are presented in Table 11. Excluding C4:0, *iso* C14:0, *cis*-9, *trans*-11 CLA, C22:0, and total PUFA, differences in milk fat proportions were detected

for all FA measured. Short- and medium-chain FA (< C16:0), odd-branched chain FA (OBCFA), saturated FA (SFA), and n-6 concentrations decreased with GFLAX supplementation. Long-chain FA (with the exception of C18:2, n-6; C18:3, n-6; C20:2, C20:3, n-6; C20:4, n-6, which decreased), MUFA, linolenic acid, and n-3 showed increases in concentration with the 10% GFLAX treatment. Trends for decreases in C15:0, *iso* C15:0, C16:0, and increases in *trans*-11 C18:1 due to GFLAX treatment were observed.

Income over feed cost

Income over feed cost analysis results are shown in Table 12. Cows on the 0% GFLAX diet had a higher IOFC ($5.53/cow/d) compared with cows fed the 10% GFLAX ($4.61/cow/d).

DICUSSION

Dry matter intake, milk production and milk composition

Pasture intake was higher ($P = 0.01$) when estimated by difference and showed a trend ($P = 0.07$) for higher estimated pasture intake, as estimated with all three methods (IVDMD, AIA, and iADF) using chromium oxide, when cows received the 10% GFLAX diet. However, this did not translate to a difference in total DMI because cows on the control diet consumed more ($P = 0.01$) DM as TMR. Although GFLAX was included in the diet at a level of 10% of diet DM, total DMI was not affected by supplementation. The similar DMI may be attributed to similar NE_L concentration values and positive energy balance in both diets indicating that energy needs were met for cows regardless of dietary treatment. Moreover, the NRC (2001) predicted DMI was also similar.

Gonthier et al. (2004) supplemented GFLAX to confined lactating dairy cows at a similar level (12.7% of diet DM) to that used in the present experiment and did not observe a decrease in DMI compared with the control treatment. Extruded flaxseed, fed at 9% of dietary DM to lactating dairy cows in combination with a high forage basal diet (60% of DM) did not decrease DMI (Neveu et al., 2013).

Resende et al. (2014, unpublished data) observed a significant linear decrease in DMI in dairy cows fed incremental amounts (0, 5, 10, or 15% of diet DM) of GFLAX. However, the difference in total DMI (-1.1 kg/d) between the 0 and 10% GFLAX diet was relatively small. Decreases in DMI (-1.5 kg/d) were also reported by Lerch et al. (2012a) when lactating cows consuming grass-based diets during the winter and grazing during the summer were supplemented with extruded flaxseed for 2 consecutive lactations. Throughout the literature, variations in DMI have been reported and may be

attributed to flaxseed processing, basal diet composition, level of inclusion in the diet, and forage-to-concentrate ratio.

Milk production as well as milk component yields and concentrations were not affected by feeding GFLAX. These results are similar to those of Gonthier et al. (2004) in which a non-significant decrease of 1.8 kg milk/d was observed when cows were supplemented with GFLAX, micronized flaxseed, or extruded flaxseed at 12.6% of diet DM compared with the control diet. In addition, Gonthier et al. (2004) did not observe any effects of flaxseed supplementation on milk fat, protein, or lactose concentrations. Petit and Côrtes (2010) included GFLAX at 7.2% of dietary DM for lactating cattle fed TMR and no differences due to diet were observed for milk production or milk components. The lack of change in milk production in the present study is likely explained by similar DMI across treatments.

Energy corrected milk ($P = 0.23$) and 4% FCM ($P = 0.23$) in cows receiving 0 or 10% GFLAX averaged 21.2 vs. 19.9 kg/d and 19.5 vs. 18.3 kg/d, respectively. Feed efficiencies, calculated based on milk yield, ECM, and 4% FCM were not affected by the dietary treatments. These results can be explained by similar DMI, milk production, and yields and concentrations of milk components between diets.

Differences in milk production as well as some milk component concentrations and milk component yields across months were observed. Milk production was highest in August followed by June, July, and September and averaged 19.25, 18.29, 17.44 and 16.50 kg/d, respectively. Higher milk production in August was likely due to higher DMI. In addition, August pasture allowance was higher by at least 11 kg DM/cow/d than all other months and increased pasture allowance is correlated with higher DMI and

higher milk production (Stockdale, 2000; Bargo et al., 2003). Decreased DMI in June was attributed to lower quality baleage, which averaged 8.5% lower CP, 5.3% higher ADF, and 8.5% higher NDF than the remaining months. In addition, pasture allowance was lower in June than in August and September. Despite the lower DMI observed in June, milk production was higher in June than in July and September. This can partially be explained by the cows being earlier in lactation than the following months. Pasture herbage allowance was very low in July compared to the other three months and may also have contributed to the lower milk production. Another contributing factor for higher milk production in both August and June was a lower pasture CP concentration and a corresponding decrease in PUN concentrations was observed. In July and September, pasture samples collected for botanical composition indicated that 23.5 and 17.1% of pasture was composed by legumes. The high legume content directly translated to a higher pasture CP of 21.3 and 22.7% for July and September, respectively. Higher CP in the pasture coupled with higher CP baleage (average +8.5% CP compared to June) may have led to inefficient rumen microbial utilization of CP (Colmenero and Broderick, 2006) and generated high levels of blood urea (Hodgson and Brookes, 1999). Significantly higher concentrations of MUN and PUN during July and September support this hypothesis. Excess rumen degradable protein is converted to ammonia, which is absorbed through the rumen wall and finally converted to urea in the liver (NRC, 2001). It is noteworthy that ureagenesis is an energy-costly reaction (NRC, 2001) that has a potential cost of 1 ATP for conversion of each N atom to urea (Dijkstra et al., 2005). In addition, the cost of protein deamination to provide an extra N atom for urea formation,

while not directly involved in the reaction, must be taken into account (Dijkstra et al., 2005).

Milk yield efficiency was increased (P <0.001) in June and August compared to the other two months. Higher efficiency in August was likely due to high milk production and similar DMI to July and September. Higher efficiency in June may be attributed to lower DMI (P <0.01) than the other three months combined with the second highest milk production. Although milk fat yield did not differ among months, 4% FCM efficiency decreased throughout the study. The highest efficiency observed in June is likely due to low DMI with relatively high milk production. As the study progresses, DMI remains the same between the months of July to September, however, milk production fluctuates, leading to the differences in 4% FCM efficiency.

Blood parameters, BW, and BCS

Plasma NEFA, BW, BCS and serum cortisol were not affected by dietary treatment. The similar plasma NEFA concentrations, BW, and BCS between dietary treatments in the current study can be explained by similar NE_L values and similar DMI between treatments. Petit and Côrtes (2010) observed similar results when lactating cows receiving TMR were supplemented with GFLAX (7.2% of diet DM). Lerch et al. (2012a) fed extruded flaxseed (+2.5 to 3.0% additional oil in ration DM compared with control diet) to cows on grass-based diets for 2 consecutive lactations and reported that dietary treatment did not affect BW, BCS, or plasma NEFA concentrations between cows receiving extruded flaxseed or the control diet. Gonthier et al. (2005) observed similar DMI with increased plasma NEFA concentrations in lactating cows supplemented with

ground, micronized, or extruded flaxseed (12.6% of diet DM) compared with those in the control diet. The authors suggested the increase in plasma NEFA was due to increased dietary FA intake. In the present study, ether extract was higher (+2.6% of DM) in the 10% GFLAX treatment, however, it did not impact plasma NEFA concentration as suggested by Gonthier et al. (2005).

Apparent total tract digestibility of nutrients

Apparent total tract DM digestibility was significantly lower for cows on the 10% GFLAX diets when calculated using chromium oxide and IVDMD or AIA and demonstrated a trend to be lower when calculated using chromium oxide and iADF. Only AIA methodology indicated decreased ADF digestibility in the 10% GFLAX diets. Decreased apparent total tract nutrient DM and ADF digestibility suggests that increased fat in the diet may have marginally affected ruminal function. Maia et al. (2007) evaluated metabolism of PUFA on the function of ruminal microbes in vitro. Growth was inhibited in 5 of the 26 bacterial species studied when linoleic acid was dosed at 50 µg/mL and damage to cell membranes was noted in all species. When linolenic acid was added to 24 different species, 11 demonstrated decreased growth. Linolenic acid was shown to have higher toxicity to cell growth than linoleic acid (Maia et al., 2007). Decreased DM digestibility with flaxseed supplementation may be attributed to the influences of increased fat (+ 2.6% of DM) in the flaxseed treatment and higher levels of linolenic acid. Total fat in the diet was slightly higher than commonly recommended in the diets of dairy cows. The high level of PUFA with the 10% GFLAX in the current

study may have impaired bacterial growth and contributed to reduced ruminal fermentation.

All 3 methodologies indicated that OM digestibility was significantly higher when cows received the 10% GFLAX diet. Two methods (IVDMD; $P = 0.10$ and iADF; $P = 0.05$) indicated that NDF digestibility was higher in the 10% GFLAX treatment. Both IVDMD ($P = 0.05$) and iADF ($P = 0.01$) indicated higher CP digestibility in cows receiving the 10% GFLAX treatment. The trend for higher pasture intake may partially explain the increased NDF and CP digestibility with the 10% GLFAX treatment. The average concentration of pasture NDF and ADF was lower while that of pasture CP was higher than the NDF, ADF, and CP of the baleage.

Similar to the results of the current study, Gonthier et al. (2004) observed increases in total tract OM digestibility when lactating cows consuming TMR were supplemented with GFLAX (12.6% of DM). In agreement with the IVDMD and iADF methodology used in the current study, higher total tract NDF and CP digestibility was observed for cows receiving flaxseed compared to those in the control group (Gonthier et al., 2004). Although total tract ADF digestibility was not affected by treatment, ruminal ADF digestibility was decreased by flaxseed supplementation in the study of Gonthier et al. (2004).

Doreau et al. (2009) did not observe differences in apparent total tract digestibility of DM, OM, NDF, or ADF when various forms of flaxseed (rolled, extruded, oil) were supplemented to dry cows in confinement. However, Martin et al. (2008) noted decreases in OM and fiber digestibility when the control diet was compared with diets supplemented with various forms of flaxseed (whole, extruded, oil).

Scholljegerdes and Kronberg (2010) fed GFLAX (0.18% of BW) or cracked corn/soybean meal (0.32% of BW) to beef cattle grazing summer native range in the northern Great Plains and observed significantly lower total tract apparent digestibility of OM and NDF consuming GFLAX than cattle consuming the corn-based supplement. Continuous culture work (Soder et al., 2012) also indicates that supplementation with GFLAX at 0, 5, 10, or 15% of the diet, linearly decreased total tract DM, OM, and NDF apparent digestibility. Conversely, when GFLAX, canola, or sunflower seed were supplemented at 10% of diet DM with an herbage-based diet in continuous culture (Soder et al., 2013) apparent digestibility of DM, OM, NDF, and CP were not significantly different between diets.

A final factor to be considered in the interpretation of apparent total tract digestibility of nutrients is that each method of digestibility estimation includes challenges that can lead to disagreement among methodologies. The variation across methods in the current study illustrates that apparent total tract digestibility can be largely influenced by the chosen methodology and conclusions should be drawn with caution. Based on the apparent total tract digestibility reported in the current study and others reported in the literature, the form of flaxseed, the amount supplied, and the basal diet affect the digestibility of the diet.

Ruminal pH and volatile fatty acids

Lodge-Ivey et al. (2009) compared the collection method on ruminal sample characteristics using an oral lavage tube and ruminal cannula with cattle and sheep. The authors noted that after technicians gain experience, saliva contamination can be

eliminated and results indicated that total VFA, molar proportions of individual VFA, and ruminal ammonia concentrations were not affected by sampling method. Ruminal pH in the current study was not affected by dietary treatment, however, these results should be interpreted with caution. Although an attempt to minimize saliva contamination during collection was made, saliva contamination was noted in at least two samples.

A shift for lower lipogenic to glucogenic ratios of VFA production was observed for cows on the 10% GFLAX diet. This shift may be attributed to the previously mentioned toxicity of PUFA on rumen microbial species (Maia et al., 2007). Of the 25 species dosed with linoleic and linolenic acid in culture, growth of 2 cellulolytic fermenter species and 3 butyrate-producing species was inhibited. Inhibitory effects of PUFA in the 10% GFLAX treatment on the ruminal microbes may have led to decreased digestion of fiber. Acetic and butyric acid (both lipogenic building blocks in the mammary gland) are 2 main products of fiber digestion via cellulolytic fermenting bacterial species. The work of Maia et al. (2007) also supports the trend for decreased acetic and increased propionic acid proportions in the 10% GFLAX treatment.

The results of the current study agree with Gonthier et al. (2004) in which ruminal pH and total VFA were not affected by dietary treatments of ground, micronized, or extruded flaxseed fed at 12.7% of dietary DM. The ratio of acetic to propionic acid was significantly higher in the control diet (no flaxseed) than in the 3 diets containing flaxseed, similar to the results of the current study. Also similar to the current study, cows receiving the flaxseed supplemented diets had higher proportions of propionic acid and lower proportions of acetic acid than cows on the control diet. However, the authors reported that the changes in molar proportions of acetic and propionic acid may have

been due to higher forage intake (+8% of DM) in the control diet, rather than the flaxseed supplementation. The effects of GFLAX supplementation on ruminal microbes in relation to the current study is further supported by the work of Soder et al. (2012). When GFLAX was fed at 0, 5, 10, or 15% in continuous culture fermenters, the molar proportion of propionic acid increased linearly. The ratio of acetic to propionic acid and the molar proportion of butyric acid also decreased linearly (Soder et al., 2012).

Methane and carbon dioxide production

Enteric CH_4 and CO_2 production were not affected by dietary treatment. Enteric CH_4, expressed per unit of DMI or OM intake, was not affected by supplementation with GFLAX. All of the factors were affected by month, likely due to changes in pasture composition and maturity, baleage quality, and fluctuations in DMI. One possible explanation for the lack of effect on greenhouse gas emissions due to GFLAX supplementation could be the highly digestible nature of the pasture consumed. One proposed mechanism of action for oilseed CH_4 mitigation is through toxicity to cellulolytic ruminal bacteria and inhibition of fiber digestibility (Beauchemin et al., 2009). However, in the present study, fiber digestibility was not compromised by GFLAX supplementation. Another proposed mechanism of action is by decreasing DMI with a combined increase in milk production to increase efficiency (Hristov et al., 2013). However, feed efficiency was not increased with GFLAX supplementation in this study. Intensive management of pastures to maintain forage immaturity and decrease fiber content can be utilized as a CH_4 mitigation technique (Hristov et al., 2013). Cows in the

present study were maintained on intensively managed pastures with an approximate rotation schedule of 3 to 4 wk, which maintained pasture at the desired maturity level.

The results of this study are in contrast to those of Resende et al. (2013) in which GFLAX was fed at 0, 5, 10, or 15% of the diet DM and a linear reduction in both DMI and enteric CH_4 was observed. An 18% decrease in enteric CH_4 was observed by Beauchemin et al. (2009) when dairy cows fed a TMR diet supplemented with crushed flaxseed was compared with the control diet. Martin et al. (2008) also observed decreases in CH_4 production when dairy cows consuming TMR diets supplemented with various forms of flaxseed were compared with a control diet. Results of the Martin et al. (2008) study indicated that crude flaxseed, extruded flaxseed, and flaxseed oil decreased CH_4 emissions by 12, 38, and 64%, respectively, when compared with the control diet. The authors suggested that the reduction was due to reduced NDF digestibility which disagrees with the results of the current study in which 10% GFLAX increased NDF digestibility. Also contrary to the results of the current study, a linear reduction in CH_4 production was observed when GFLAX was fed to fermenters in continuous culture at 0, 5, 10, or 15% of diet DM (Soder et al., 2012).

Jiao et al. (2014) did not observe differences in daily CH_4 emissions when varying levels of a corn/wheat/soybean meal supplement was provided to lactating dairy cows on pasture. However, decreases in CH_4/DMI and CH_4/ECM were observed during at least 1 of the 4 treatment periods when levels of supplement in the diet were increased. These results indicate that although concentrate supplementation can reduce CH_4 emissions during the grazing season, the results may not be consistent throughout the study and can

be influenced by similar factors observed in the current study such as stage of lactation, pasture species composition, and weather conditions.

O'Neill et al. (2012) observed that DMI differences between grazing groups can lead to differences in CH_4 production. Lactating dairy cows consuming a pTMR on a low pasture allowance emitted the highest levels of CH_4 compared with cows on either low or high pasture allowance. The cows consuming the pTMR had higher DMI than those on pasture-only. Similar DMI between treatment groups in the current study helps to explain the lack of difference in CH_4 production.

Nitrogen metabolism

Cows on 10% GFLAX treatment had higher N intake and urea N excretion than those on the 0% GFLAX treatment. A trend for higher urinary creatinine concentration was observed for cows on the 10% GFLAX diet but diet did not affect any other measured N metabolism parameters, including purine derivative excretion, N excretion, and NH_4 excretion. These results suggest that cows receiving 10% GFLAX may be more efficient in N utilization due to higher CP apparent total tract digestibility than cows on 0% GFLAX. However, higher PUN values and a trend for higher ruminal NH_4 concentration for the 10% GFLAX treatment group contradict the hypothesis of improved N efficiency. Altogether, these results indicate that N was shifted from feces to urine (in the form of urea N) in cows fed 10% GFLAX.

Rego et al. (2008) supplemented grazing dairy cows with a corn/soybean meal mixture and diets supplemented with the corn/soybean meal mixture resulted in higher PUN when compared with the diets supplemented with the corn-only concentrate, which

indicates inefficient capture of pasture N. This is similar to the results of the current study in which cows on the 10% GFLAX treatment had higher N intake due to higher CP concentration of GFLAX (+ 6.4% of DM) which caused elevated PUN. In addition, the 0% GFLAX treatment likely provided higher levels of starch facilitating better ruminal synchronization and utilization of available CP.

Corn-based supplementation to dairy cows grazing at 2 pasture allowances (high and low) can lead to more efficient N utilization as in Bargo et al. (2002). Concentrate supplementation provided increased levels of starch and decreased PUN at both pasture allowances, indicating more efficient N utilization. Additionally, allantoin and creatinine were increased by concentrate supplementation at both pasture allowances and indicated that concentrate supplementation led to more rumen microbial protein supply to the intestines. In the present study, cows on the 0% GFLAX diet demonstrated more efficient N utilization, similar to the results of Bargo et al. (2002), that can be attributed to more fermentable energy from the corn/soy mix.

Milk fatty acid composition

Ground flaxseed supplementation during the grazing season led to significantly decreased proportions of short- and medium-chain FA (<C16:0). This was supported by the ruminal VFA data that showed decreased proportions of acetic and butyric acid, the building blocks of *de novo* milk FA synthesis in the mammary gland. Significant increases in MUFA due to GFLAX supplementation were also observed. A portion of the increase in MUFA may be attributed to increased amounts of fat in the diet from the 10% GFLAX treatment. Scholljegerdes and Kronberg (2010) observed increased intestinal

flow of 10 out of 28 identified FA including MUFA, linolenic acid, and CLA, when grazing beef heifers were supplemented with GFLAX. Although the milk proportion of PUFA was not increased by GFLAX supplementation in the current study, the proportions are similar to those of other grazing studies utilizing various forms of supplementation including grass silage, corn, soybean meal, and extruded flaxseed (Rego et al., 2008; Lerch et al., 2012b).

The observed results of Lerch et al. (2012b) support the proposed mechanism that increased duodenal flow of ruminal biohydrogenation intermediates by supplying increased levels of dietary PUFA can modify milk FA. Lerch et al. (2012b) fed extruded flaxseed for 2 consecutive lactations. In the first year, milk SFA decreased and MUFA increased by extruded flaxseed supplementation. During the grazing period, linolenic acid and C20:5 n-3, were also increased by extruded flaxseed supplementation, similar to differences observed in the current study.

Mohammed et al. (2011) observed similar results when crushed flaxseed, sunflower, or canola was included the TMR of dairy cows. Saturated FA proportions were lower in oilseed supplemented diets than the control (calcium salts of long-chain FA) and MUFA were higher in the diet supplemented with crushed flaxseed compared to the control.

Vaccenic acid (i.e., *trans*-11 C18:1), which is a precursor to *cis*-9, *trans*-11 CLA, showed a trend for increased proportions in the 10% GFLAX treatment, however, it did not translate to an increase in *cis*-9, *trans*-11 CLA. When 26 strains of bacteria were dosed with linoleic acid in culture, 11 strains metabolized the FA and demonstrated

accumulations of vaccenic acid (Maia et al., 2007) and linolenic acid was metabolized by mostly the same species.

Lerch et al. (2012c) reported results similar to the current study in which CLA was not increased by flaxseed supplementation during the grazing period. It was noted by Lerch et al. (2012c) that the control diet was relatively rich in PUFA and starch and both components have been associated with accumulation of *trans*-9, *cis*-11 CLA, *trans*-10, *cis*-12 CLA, and *trans* C18:1 isomers in the rumen. Additionally, the changes in FA composition due to flaxseed supplementation were more notable during the indoor periods rather than the outdoor periods (Lerch et al., 2012c). The factors in the current study; a control diet relatively high in PUFA and starch, as well as reduced milk FA differences while on pasture, may have contributed to the similar milk CLA concentrations between diets of the current study.

When the grazing period of the second year (Lerch et al., 2012b) was compared with the first, it was found that extruded flaxseed supplementation had a reduced effect on OBCFA and total *trans* C18:1 concentrations. However, n-3 concentrations were increased to a greater extent by extruded flaxseed supplementation in the second grazing season than they had been in the first. The increase in n-3 FA observed by Lerch et al. (2012b) was similar to that found in the current study in which GFLAX supplementation increased n-3 and decreased n-6 FA proportion in milk fat.

Income over feed cost

Cows on the 0% GFLAX diet had a higher IOFC than those on the 10% GFLAX diet, $5.53 vs. $4.61, respectively. In addition, total milk income between the 2 diets was

similar, but slightly less for cows on the 10% GFLAX diet. Under the conditions of this study, the 10% GFLAX diet appears to be the less favorable option, however, if producers are offered a premium for enriched milk FA, it may contribute to offsetting the price differential. If Organic Valley were to offer a premium similar to that currently being offered for "100% grassmilk," $5/cwt higher than conventional organic milk, IOFC for cows on the 10% GFLAX diet would increase to $6.57/cow/d and make feeding GLFAX more favorable. Under the conditions of this study, any premium over $2.38/cwt would create a higher IOFC in the 10% GFLAX diet.

CONCLUSIONS

Ground flaxseed, supplemented at 10% of diet DM during the grazing season did not decrease DMI, milk production, milk component yields or concentrations. Plasma NEFA, BW, and BCS were not affected by GFLAX supplementation, indicating that GFLAX is an acceptable supplement for maintaining BW and BCS under grazing management systems. Apparent total tract digestibility results indicate that 10% GFLAX did decrease DM digestibility, however, OM and NDF digestibility were increased. Supplementation altered ruminal VFA proportions of propionic and butyric acid, likely by effects of PUFA on ruminal bacteria. Significantly higher N intake, PUN concentration, and urea N excretion by cows on the 10% GFLAX treatment indicate that reformulating the diet throughout the grazing season may be needed to improve N efficiency in cows fed GFLAX. Fatty acid composition was successfully modified by GFLAX supplementation, decreasing SFA and n-6 FA and increasing n-3 FA. This offers a promising method of manipulating milk parameters to provide more health benefits to humans in each serving of dairy. More research is needed to determine if consumer demand is present to create a "designer-milk" via flaxseed supplementation. Additional research with varying levels of GFLAX while on pasture would be useful in determining the ideal feeding rate that correlates optimum milk production with optimum FA composition.

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Table 1. Ingredient composition of the diets.

	Diet (% GFLAX[1])	
Ingredient (% of DM)	0	10
	% of DM	
Pasture, mixed mostly grass	40.00	40.00
Baleage, mixed mostly grass	25.00	25.00
Ground flaxseed	0.00	10.00
Ground corn	23.10	16.20
Roasted soybeans	2.50	2.50
Liquid molasses	1.87	1.87
Soybean meal (47.5%)	1.70	1.25
Calcium carbonate	1.15	1.15
Cane molasses	0.45	0.45
Redmond salt	0.42	0.42
Bicarbonate	0.41	0.41
Trace mineral premix	0.34	0.34
Magnesium oxide	0.24	0.24
Magnesium sulfate	0.07	0.07
Vitamin E	0.06	0.06
Yeast	0.04	0.04

[1]GFLAX; diets supplemented with ground flaxseed at 0 or 10% of diet DM.

Table 2. Ingredient composition of grain mixes.

Ingredient (% of DM)	Basal concentrate mix[1]	Corn/soy mix[2]	Chromium pellet[3]	Alfalfa pellets[4]
Alfalfa meal	--	--	4.50	100.00
Ground corn	71.15	69.45	49.56	--
Roasted soybean	10.39	30.55	10.00	--
Wheat midds	--	--	15.00	--
Barley	--	--	7.75	--
Soybean meal (47.5%)	5.37	--	2.33	--
Calcium carbonate	4.54	--	1.75	--
Molasses	2.38	--	2.38	--
Redmond salt	1.64	--	1.28	--
Sodium bicarbonate	1.63	--	1.60	--
Trace mineral premix	1.30	--	--	--
Chromium oxide[5]	--	--	1.10	--
Magnesium oxide	0.97	--	0.45	--
Magnesium sulfate	0.26	--	0.47	--
AB20[6]	--	--	0.60	--
Yeast	0.15	--	0.50	--
Vitamin A-D-E premix	--	--	0.32	--
CDF TM # 303[7]	--	--	0.26	--
Vitamin E	0.23	--	--	--
Selenium	--	--	0.13	--

[1]Basal concentrate mix was included in the TMR for both 0 and 10% GFLAX diets.
[2]Corn/soy mix was top-dressed as the control treatment.
[3]Chromium pellet (Morrison's Custom Feeds, Barnet, VT).
[4]Alfalfa pellets (Green Mountain Feeds, Bethel, VT).
[5]Chromium oxide (Cr_2O_3); (Fisher Scientific, Fair Lawn, NJ).
[6]AB20 is an advanced anti-caking agent and pelleting aid (Phibro Animal Health Corporation, Teaneck, NJ).
[7]CDF TM #303 is a trace mineral premix containing Cu, Co, Fe, Zn, and Mn (Morrison's Custom Feeds, Barnet, VT).

Table 3. Chemical composition of consumed feeds.

Item (% DM)	TMR[1]	Corn/soy mix[2]	Ground flaxseed	Chromium pellets[3]	Alfalfa pellets[4]	Baleage	Basal concentrate mix[5]
CP	13.42 ± 2.15	21.72 ± 0.82	28.13 ± 0.38	14.06 ± 0.43	13.38 ± 2.55	14.00 ± 4.34	12.96 ± 0.69
ADF	24.24 ± 1.34	4.17 ± 0.28	23.81 ± 6.33	6.74 ± 0.44	35.49 ± 2.19	35.13 ± 2.81	4.00 ± 0.60
NDF	39.37 ± 2.66	10.48 ± 0.58	33.94 ± 5.91	16.38 ± 0.49	50.84 ± 5.03	53.74 ± 7.30	10.63 ± 1.13
EE	3.42 ± 0.77	5.77 ± 0.22	30.78 ± 2.49	2.27 ± 0.42	2.21 ± 0.43	2.61 ± 0.46	3.37 ± 0.91
Ash	9.13 ± 0.99	2.96 ± 0.21	3.79 ± 0.31	10.62 ± 0.57	9.64 ± 1.91	6.88 ± 1.44	14.03 ± 0.43
Ca	1.04 ± 0.12	0.19 ± 0.05	0.30 ± 0.01	1.29 ± 0.02	--	--	--
P	0.30 ± 0.04	0.40 ± 0.02	0.57 ± 0.01	0.41 ± 0.02	--	--	--
Mg	0.52 ± 0.06	0.20 ± 0.01	0.36 ± 0.01	0.47 ± 0.03	--	--	--
K	1.74 ± 0.29	0.98 ± 0.03	0.86 ± 0.01	0.89 ± 0.02	--	--	--
S	0.27 ± 0.02	0.19 ± 0.00	0.26 ± 0.01	0.24 ± 0.01	--	--	--
Fe, ppm	270 ± 42	92 ± 18	80 ± 8	354 ± 31	--	--	--
Zn, ppm	136 ± 4	40 ± 4	55 ± 1	173 ± 80	--	--	--
Cu, ppm	27 ± 3	7 ± 1	13 ± 1	20 ± 5	--	--	--
Mn, ppm	111 ± 14	24 ± 1	46 ± 1	104 ± 7	--	--	--
Cr, ppm	2 ± 1	1 ± 1	2 ± 1	7063 ± 486	--	--	--
AIA[6]	0.55 ± 0.12	0.03 ± 0.01	0.09 ± 0.02	1.36 ± 0.13	--	--	--
IVDMD[7], % DM							
48 h	--	84.90 ± 1.22	50.43 ± 1.89	81.37 ± 0.95	55.39 ± 5.53	--	--
72 h	74.26 ± 1.05	--	--	--	--	--	--

[1]TMR is composed of baleage and basal concentrate mix but does not include top-dressed ground flaxseed or corn/soy treatments.
[2]Corn/soy mix was top-dressed as the control treatment.

[3]Chromium pellet (Morrison's Custom Feeds, Barnet, VT).
[4]Alfalfa pellets (Green Mountain Feeds, Bethel, VT).
[5]Basal concentrate mix was included in the TMR for both 0 and 10% GFLAX diets.
[6]AIA; acid insoluble ash.
[7]IVDMD; in vitro dry matter digestibility was determined using the methods described by Tilley and Terry (1963).

Table 4. Pasture management and chemical composition of hand-plucked herbage samples.

	Month				
	June	July	Aug.	Sept.	$\bar{X}$
Pasture management					
Pasture allowance, kg DM/cow/d	15.0	10.5	28.4	17.4	17.8
Area, m^2/cow/d	70	77	137	121	101
Pregrazing herbage mass, kg DM/ha	2159	1374	2072	1441	1761
Postgrazing herbage mass, kg DM/ha	1800	921	1543	1180	1361
Grass, % of DM	67.3	55.2	58.6	59.7	60.2
Legume, % of DM	10.5	23.5	14.6	17.1	16.4
Weed, % of DM	4.6	12.1	6.8	8.9	8.1
Dead, % of DM	17.6	9.2	20.1	14.3	15.3
Chemical composition					
DM, % of fresh matter	19.12	17.93	26.48	21.89	21.35
% of DM					
CP	17.65	21.30	17.99	22.70	19.91
ADF	35.77	28.85	29.70	26.26	30.15
NDF	59.32	46.67	48.76	45.43	50.05
EE	3.48	3.98	3.68	4.10	3.81
Ash	8.90	8.22	7.42	8.01	8.14
Ca	0.41	0.76	0.64	0.69	0.63
P	0.33	0.41	0.36	0.35	0.36
Mg	0.21	0.33	0.31	0.30	0.29
K	2.72	2.29	2.21	2.27	2.37
S	0.22	0.25	0.22	0.25	0.24
Fe, ppm	153	93	85	86	104
Zn, ppm	33	34	29	32	32
Cu, ppm	8	11	8	9	9
Mn, ppm	76	48	51	45	55
Cr, ppm	0.95	0.84	0.47	1.23	0.87
AIA[1]	1.44	0.79	0.90	1.03	1.04
IVDMD[2], % DMD (72 h)	70.37	73.25	71.8	74.7	72.53
NE_L[3], Mcal/kg DM	1.51	1.62	1.58	1.64	1.59

[1]AIA; acid insoluble ash.
[2]IVDMD; in vitro dry matter digestibility was determined using the methods described by Tilley and Terry (1963).
[3]NE_L; net energy of lactation was estimated using the NRC model (NRC, 2001) and chemical composition of hand-plucked herbage samples.

Table 5. NRC (2001) evaluation of consumed diets.

	Diet (% of GFLAX[1])	
Item[2]	0	10
NDF, % DM	37.6	39.2
Forage NDF, % DM	34.4	33.5
ADF, % DM	22.8	24.3
NFC, % DM	37.0	31.8
ME[3], Mcal/kg DM	2.50	2.62
EE, % DM	3.9	6.5
DCAD[4], mEQ/kg	275	271
NE_L[5], Mcal/kg DM	1.58	1.63
NE_L required, Mcal/d	25.5	24.7
NE_L supplied, Mcal/d	26.4	28.4
NE_L balance, Mcal/d	0.9	3.6
MP[6] required, g/d	1538	1515
MP supplied, g/d	1652	1829
MP balance, g/d	115	314
DM intake-actual[7], kg/d	16.8	16.9
DM intake-predicted, kg/d	15.6	15.1
NE_L allowable milk, kg/d	19.3	22.2
MP allowable milk, kg/d	20.4	23.5
Actual milk, kg/d	18.2	17.5
CP, % DM	16.0	17.5
RDP[8], % DM	10.7	11.3
RUP[9], % DM	5.3	6.2
RDP balance, g/d	134	184
RUP balance, g/d	151	406

[1]GFLAX; diets supplemented with ground flaxseed at 0 or 10% of diet DM.
[2]Values predicted from previously described diets and animal inputs from each treatment using the NRC (2001) model.
[3]ME; metabolizable energy.
[4]DCAD; dietary cation-anion difference.
[5]NE_L; net energy of lactation.
[6]MP; metabolizable protein.
[7]Actual DMI estimated using chromium oxide and iADF methodology.
[8]RDP; rumen degradable protein.
[9]RUP; rumen undegradable protein.

Table 6. Fatty acid composition of ground flaxseed, corn/soy mix[1], TMR[2], and pasture.

	Ground flaxseed	Corn/soy mix	TMR	Pasture
FA	---------- g/100 of total feed FA ----------			
14:0	0.05	0.07	0.33	0.48
16:0	5.26	13.14	18.06	15.17
16:1	0.12	0.12	0.24	0.26
17:0	0.07	0.09	0.19	0.20
18:0	4.83	3.15	3.54	1.70
cis-9 18:1	24.48	21.57	17.44	2.25
cis-11 18:1	0.62	1.12	1.04	0.39
cis-9, *cis*-12 18:2 (LA)[3]	15.84	51.20	31.69	15.49
cis-6, *cis*-9, *cis*-12 18:3 (GLA)[4]	ND*	ND	0.03	ND
cis-9, *cis*-12, *cis*-15 18:3 (ALA)[5]	42.20	5.05	15.57	46.64
20:0	0.20	0.37	0.78	0.65
20:1	0.18	0.23	0.27	0.07
20:2	0.04	0.04	0.06	0.16
22:0	0.26	0.31	0.79	0.75
20:3	0.01	ND	ND	0.08
24:0	0.16	0.21	0.76	0.72
24:1	4.93	0.10	0.37	0.20
22:4	0.03	2.42	0.12	0.15
Unidentified	0.70	0.80	8.72	14.64

*ND; not detected.
[1]Corn/soy mix was top-dressed as the control treatment.
[2]TMR is composed of baleage and basal concentrate mix but does not include top-dressed ground flaxseed or corn/soy treatments.
[3]LA; linoleic acid.
[4]GLA; γ-linolenic acid.
[5]ALA; α-linolenic acid.

Table 7. Milk yield, concentrations and yields of milk components, feed efficiency, body weight (BW), body condition score (BCS), and plasma concentrations of urea N (PUN), nonesterified fatty acids (NEFA), and cortisol in cows fed 0 or 10% GFLAX[1].

	Diet (% GFLAX)*			Month**					*P*-value[2]		
Item	0	10	SEM	June	July	Aug.	Sept.	SEM	T	M	T×M
Total DMI[3], kg/d	16.79	16.86	0.19	16.33^{b}	16.90^{a}	17.11^{a}	16.97^{a}	0.19	0.79	<0.01	0.98
TMR intake, kg/d	10.92	10.53	0.09	9.98^{d}	10.64^{c}	11.30^{a}	10.99^{b}	0.09	0.01	<0.001	0.01
Pasture intake, kg/d[4]	5.88	6.32	0.16	6.35^{a}	6.26^{a,b}	5.81^{c}	5.99^{b,c}	0.16	0.07	0.01	0.98
Pasture intake, kg/d[5]	6.78	7.17	0.09	7.72^{a}	7.06^{b}	6.41^{d}	6.72^{c}	0.09	0.01	<0.001	0.01
Milk yield[6], kg/d	18.20	17.54	0.62	18.29^{b}	17.44^{c}	19.25^{a}	16.50^{d}	0.49	0.45	<0.001	0.94
Milk fat[7], %	4.30	4.15	0.15	4.10	4.32	4.24	4.21	0.15	0.50	0.55	0.84
Milk fat, kg/d	0.79	0.75	0.03	0.77	0.81	0.78	0.73	0.03	0.27	0.16	0.72
Milk protein, %	3.44	3.49	0.07	3.18^{c}	3.42^{a}	3.47^{a}	3.79^{b}	0.05	0.66	<0.001	0.93
Milk protein, kg/d	0.64	0.62	0.02	0.60^{b}	0.64^{a}	0.63^{a,b}	0.66^{a}	0.02	0.52	0.03	0.97
Milk lactose, %	4.77	4.78	0.02	4.79^{a}	4.73^{c}	4.80^{a}	4.77^{a,b}	0.02	0.65	0.01	0.17
Milk lactose, kg/d	0.90	0.86	0.03	0.91	0.89	0.88	0.84	0.03	0.36	0.07	0.98
Milk SNF[8], %	9.12	9.23	0.08	8.90^{d}	9.06^{c}	9.21^{b}	9.53^{a}	0.06	0.31	<0.001	0.89
Milk SNF, kg/d	1.72	1.65	0.06	1.68	1.70	1.68	1.67	0.05	0.42	0.91	0.99
Milk TS[9], %	13.48	13.32	0.17	13.00^{b}	13.42^{a}	13.45^{a}	13.73^{a}	0.16	0.52	<0.01	0.87
Milk TS, kg/d	2.52	2.39	0.08	2.45	2.51	2.46	2.39	0.07	0.28	0.48	0.90
4% FCM[10], kg/d	19.54	18.31	0.70	19.15	19.63	18.99	17.93	0.64	0.23	0.08	0.79
ECM[11], kg/d	21.16	19.93	0.70	20.50	21.20	20.60	19.89	0.64	0.23	0.29	0.82
MUN, mg/dL	14.54	13.33	0.49	11.84^{c}	15.01^{b}	11.77^{c}	17.13^{a}	0.41	0.10	<0.001	0.03
SCS[12]	2.4	2.2	0.30	2.1^{b}	3.0^{a}	1.9^{b}	2.3^{b}	0.28	0.68	<0.01	0.53
Milk yield/DMI, kg/kg	1.08	1.05	0.05	1.13^{a}	1.03^{b}	1.13^{a}	0.98^{c}	0.04	0.71	<0.001	0.36
4% FCM/DMI, kg/kg	1.13	1.11	0.05	1.17^{a}	1.14^{b}	1.11^{c}	1.05^{d}	0.04	0.72	0.04	0.97
ECM/DMI, kg/kg	1.22	1.20	0.05	1.26	1.23	1.20	1.17	0.04	0.77	0.20	0.97
BW, kg	431.6	429.5	5.35	421.3^{c}	427.3^{b}	431.9^{b}	441.7^{a}	3.99	0.79	<0.001	0.60
BCS[13]	3.01	2.97	0.05	2.97^{b,c}	2.90^{c}	3.01^{a,b}	3.08^{a}	0.05	0.66	0.02	0.18
PUN, mg/dL	12.67	14.42	0.45	10.04^{d}	14.02^{b}	12.86^{c}	17.26^{a}	0.44	0.01	<0.001	0.82
Plasma NEFA, mEq/L	217.4	204.2	11.16	298.2^{a}	245.0^{a}	136.3^{c}	163.7^{b}	22.39	0.32	<0.001	0.60
Serum cortisol, ng/mL	63.78	66.95	8.96	67.88	67.63	63.91	62.04	7.89	0.80	0.89	0.35

*n = 10, **n = 20.
[1]GFLAX; diets supplemented with ground flaxseed at 0 or 10% of diet DM.
[2]T = control vs. flaxseed, M = month, T×M = interaction of treatment with month.
[3]Total DMI calculated with the use of the external fecal marker chromium oxide and indigestible acid detergent fiber.
[4]Pasture intake estimated using the external fecal marker chromium oxide and indigestible acid detergent fiber.
[5]Pasture intake = [Expected DMI (kg/d)] – [TMR DMI (kg/d)].
[6]Milk yield is based on 7 d sampling period average of recorded milk weights.
[7]Milk component percentages and yields are based on 2 d milk sampling average.
[8]SNF; solids non-fat.
[9]TS; total solids.
[10]4% FCM; 4% fat corrected milk = [0.4 × milk yield (kg/d)] + [15 × fat yield (kg/d)] (Gaines and Davidson, 1923).
[11]ECM; energy corrected milk = [0.327 × milk yield (kg/d)] + [12.95 ×fat yield (kg/d)] + [7.2 × protein yield (kg/d)] (Orth, 1992).
[12]SCS; somatic cell scores were determined from somatic cell counts using a linear scoring system (DRMS, 2014).
[13]BCS; body condition score was evaluated using the guidelines of Wildman et al. (1982).

Table 8. Apparent total tract digestibility of cows fed 0 or 10% GFLAX[1] during the grazing season. Calculated with the use of the external fecal marker chromium oxide[2] in combination with IVDMD[3], AIA[4], or iADF[5] methodology.

	Diet (% GFLAX)*			Month**					*P*-value[6]		
Item	0	10	SEM	June	July	Aug.	Sept.	SEM	T	M	T×M
IVDMD											
Total DMI, kg/d	16.79	16.86	0.19	16.33^{b}	16.90^{a}	17.11^{a}	16.97^{a}	0.19	0.79	<0.01	0.24
Pasture intake, kg/d	5.88	6.32	0.16	6.35^{a}	6.26^{a}	5.81^{b}	5.99^{a,b}	0.16	0.07	0.01	0.98
DM digestibility, %	65.01	62.47	0.63	61.13^{c}	63.04^{b}	66.02^{a}	64.76^{a}	0.60	0.01	<0.001	0.73
OM digestibility, %	55.68	56.90	0.31	57.99^{a}	58.31^{a}	52.13^{c}	56.72^{b}	0.32	0.01	<0.001	0.02
NDF digestibility, %	57.30	59.14	0.76	57.21^{b,c}	56.30^{c}	60.14^{a}	59.24^{a,b}	0.95	0.10	0.01	0.56
ADF digestibility, %	53.28	52.81	0.96	52.64^{b}	52.76^{b}	54.83^{a}	51.95^{b}	0.91	0.73	0.02	0.05
CP digestibility, %	62.81	65.44	0.91	58.87^{a}	65.55^{b}	65.00^{b,c}	67.08^{c}	0.81	0.05	<0.001	0.27
AIA											
Total DMI, kg/d	16.82	16.89	0.19	16.35^{b}	16.88^{a}	17.05^{a}	17.05^{a}	0.19	0.79	<0.01	0.24
Pasture intake, kg/d	5.91	6.35	0.16	6.37^{a}	6.24^{a}	5.75^{b}	6.15^{a}	0.16	0.07	0.01	0.97
DM digestibility, %	76.78	73.43	1.07	74.09^{b}	79.64^{a}	70.70^{c}	76.01^{b}	0.94	0.04	<0.001	0.07
OM digestibility, %	55.72	56.94	0.31	58.03^{a}	58.28^{a}	52.04^{c}	56.98^{b}	0.32	0.01	<0.001	0.02
NDF digestibility, %	71.62	71.22	1.10	71.50^{b}	76.12^{a}	65.67^{c}	72.39^{b}	1.09	0.80	<0.001	0.13
ADF digestibility, %	71.41	66.65	1.25	70.13^{b}	74.96^{a}	62.42^{c}	68.61^{b}	1.14	0.01	<0.001	0.16
CP digestibility, %	75.27	75.92	1.11	72.43^{c}	81.27^{a}	70.74^{d}	77.95^{b}	1.09	0.66	<0.001	0.33
iADF											
Total DMI, kg/d	16.79	16.86	0.19	16.33^{b}	16.90^{a}	17.11^{a}	16.97^{a}	0.19	0.79	<0.01	0.24
Pasture intake, kg/d	5.88	6.32	0.16	6.35^{a}	6.26^{a,b}	5.81^{c}	5.99^{b,c}	0.16	0.07	0.01	0.98
DM digestibility, %	62.64	60.80	0.66	54.53^{d}	61.50^{c}	64.35^{b}	66.49^{a}	0.72	0.07	<0.001	0.47
OM digestibility, %	55.68	56.90	0.31	57.99^{a}	58.32^{a}	52.13^{c}	56.72^{b}	0.32	0.01	<0.001	0.02
NDF digestibility, %	54.62	57.35	0.94	49.83^{d}	54.61^{c}	58.18^{b}	61.34^{a}	1.03	0.05	<0.001	0.85
ADF digestibility, %	50.42	50.84	0.74	44.51^{c}	50.84^{b}	52.65^{a,b}	54.52^{a}	0.87	0.70	<0.001	0.93
CP digestibility, %	59.79	63.16	0.82	51.31^{c}	63.23^{b}	63.35^{b}	68.01^{a}	0.87	0.01	<0.001	0.01

*n = 10, **n = 20.

[1]GFLAX; diets supplemented with ground flaxseed at 0 or 10% of diet DM.
[2]Chromium oxide; Cr_2O_3 was dosed in a pelleted form twice daily with a target intake of 10 g/d.
[3]IVDMD; in vitro dry matter digestibility was determined using the methods described by Tilley and Terry (1963); concentrates and forages were incubated for 48 and 72 h, respectively.
[4]AIA; acid insoluble ash.
[5]iADF; indigestible acid detergent fiber.
[6] T = control vs. flaxseed, M = month, T×M = interaction of treatment with month.

Table 9. Ruminal pH†, volatile fatty acid concentrations† and proportions†, and enteric CH_4 and CO_2 production of cows fed 0 or 10% of GFLAX[1] during the grazing season.

	Diet (% GFLAX)*			Month**					*P*-value[2]		
Item	0	10	SEM	June	July	Aug.	Sept.	SEM	T	M	T×M
pH	6.87	6.93	0.08	7.04^{a}	6.93^{a,b}	6.77^{c}	6.87^{b,c}	0.07	0.60	0.02	0.83
A:P[3]	4.08	3.67	0.08	4.58^{a}	3.93^{b}	3.55^{c}	3.43^{c}	0.07	<0.01	<0.001	0.32
A+B:P[4]	4.79	4.29	0.09	5.24^{a}	4.63^{b}	4.22^{c}	4.07^{c}	0.09	<0.01	<0.001	0.36
mmol/L											
Acetic acid	52.87	49.25	2.56	46.58^{b}	50.77^{a,b}	56.87^{a}	50.03^{b}	2.76	0.35	0.07	0.72
Propionic acid	13.27	13.64	0.54	10.18^{c}	12.95^{b}	16.05^{a}	14.64^{a,b}	0.67	0.64	<0.001	0.83
Butyric acid	9.46	8.58	0.49	6.71^{c}	9.11^{b}	10.83^{a}	9.42^{b}	0.50	0.24	<0.001	0.75
Isobutyric acid	0.81	0.76	0.04	0.52^{c}	0.78^{b}	0.93^{a}	0.92^{a}	0.04	0.40	<0.001	0.56
Valeric acid	0.89	0.85	0.05	0.51^{c}	0.85^{b}	1.11^{a}	1.02^{a,b}	0.06	0.59	<0.001	0.83
Isovaleric acid	0.60	0.53	0.03	0.35^{c}	0.51^{b}	0.70^{a}	0.71^{a}	0.04	0.20	<0.001	0.46
Total VFA	77.89	73.63	3.62	64.85^{c}	74.98^{b}	86.48^{a}	76.73^{b}	3.98	0.43	0.01	0.76
mol/100 mol											
Acetic acid	68.00	67.20	0.25	71.81^{a}	67.71^{b}	65.71^{c}	65.17^{c}	0.29	0.06	<0.001	0.54
Propionic acid	16.96	18.43	0.23	15.75^{b}	17.30^{b}	18.62^{a}	19.09^{a}	0.26	<0.01	<0.001	0.88
Butyric acid	12.10	11.51	0.15	10.31^{b}	12.12^{a}	12.50^{a}	12.29^{a}	0.15	0.02	<0.001	0.42
Ruminal NH_4, mg/dL	11.31	12.83	0.49	6.97^{c}	10.51^{b}	15.29^{a}	15.52^{a}	0.62	0.06	<0.001	0.16
Enteric CO_2, g/cow/d	8705	9012	158	8459^{c}	7839^{d}	9867^{a}	9269^{b}	168	0.18	<0.001	0.97
Enteric CH_4, g/cow/d	314	303	8.22	308^{b}	287^{c}	341^{a}	298^{b,c}	7.98	0.36	<0.001	0.81
CH_4/DMI, g/kg	18.6	18.1	0.41	19.0^{a}	17.0^{b}	19.9^{a}	17.4^{b}	0.45	0.38	<0.001	0.73
CH_4/OM intake, g/kg	16.9	16.8	0.24	16.3^{b}	16.9^{a}	17.2^{a}	17.0^{a}	0.23	0.97	0.01	0.35

†Covariate values were not used in the statistical model for ruminal pH or VFA concentrations or proportions.

*n = 10, **n = 20.

[1] GFLAX; diets supplemented with ground flaxseed at 0 or 10% of diet DM.

[2] T = control vs. flaxseed, M = month, T×M = interaction of treatment with month.

[3]A:P; acetic acid to propionic acid ratio.

[4]A+B:P; acetic acid plus butyric acid to propionic acid ratio.

Table 10. N intake, urinary concentration and excretion of purine derivatives (PD = allantoin plus uric acid), N excretion (total manure[1]) and urea N, and urinary ammonia (NH_4) of cows fed 0 or 10% GFLAX[2] during the grazing season.

	Diet (% GFLAX)*			Month**					*P*-value[3]		
Item	0	10	SEM	June	July	Aug.	Sept.	SEM	T	M	T×M
N intake, g/d	446.30	470.42	5.76	381.37^{d}	481.34^{b}	461.90^{c}	508.83^{a}	5.77	0.01	<0.001	0.31
Creatinine, mmol/L	2.26	2.48	0.08	2.72^{a}	2.31^{b}	2.33^{b}	2.12^{b}	0.10	0.08	<0.001	0.31
PD[4], mmol/L	5.76	6.23	0.24	6.11^{b}	7.24^{a}	5.16^{c}	5.48^{b,c}	0.35	0.17	<0.001	0.66
PD:creatinine ratio	2.64	2.51	0.10	2.28^{b,c}	3.19^{a}	2.24^{c}	2.59^{b}	0.14	0.31	<0.001	0.27
Allantoin, mmol/d	264.61	258.35	9.76	231.23^{b}	319.68^{a}	232.07^{b}	262.96^{b}	15.14	0.65	<0.001	0.47
Uric acid, mmol/d	19.74	21.17	1.85	15.68^{c}	23.62^{b}	13.56^{c}	28.97^{a}	2.01	0.58	<0.001	0.18
Allantoin:Uric Acid	18.64	16.85	1.92	22.14^{a,b}	14.45^{b}	24.22^{a}	10.17^{c}	3.82	0.36	<0.001	0.57
PD[4], mmol/d	285.00	279.75	9.97	246.66^{c}	343.73^{a}	246.87^{c}	292.24^{b}	15.35	0.71	<0.001	0.34
N excretion, g/d	318.65	340.68	9.60	284.69^{c}	403.66^{a}	288.49^{c}	341.81^{b}	11.08	0.12	<0.001	0.17
N excretion, % of N intake	71.59	72.38	1.50	74.65^{b}	83.98^{a}	62.49^{c}	66.84^{c}	2.17	0.72	<0.001	0.58
Urea N excretion, g/d	178.64	201.66	7.15	133.70^{c}	178.62^{b}	194.16^{b}	254.12^{a}	6.97	0.03	<0.001	0.97
Urea N excretion, % of N intake	39.55	42.56	1.43	35.13^{c}	37.14^{c}	42.02^{b}	49.93^{a}	1.42	0.15	<0.001	0.95
NH_4 excretion, g/d	0.35	0.36	0.03	0.36^{b}	0.47^{a}	0.26^{c}	0.32^{b,c}	0.03	0.91	<0.001	0.73
NH_4, % of N intake	0.08	0.08	0.01	0.09^{a}	0.10^{a}	0.06^{b}	0.06^{b}	0.01	0.76	<0.001	0.67

*n = 10, **n = 20.

[1]Total manure N excretion = fecal + urinary N excretion.

[2]GFLAX; diets supplemented with ground flaxseed at 0 or 10% of diet DM.

[3]T = control vs. flaxseed, M = month, T×M = interaction of treatment with month.

[4]PD; purine derivatives calculated with PD:creatinine excretion ratio (Chizzotti et al., 2008) using a standard value of 29 mg creatinine excretion/kg BW (Valadares et al., 1999).

Table 11. Milk fatty acid composition (% of total FA) of cows fed 0 or 10% GFLAX[1] during the grazing season.

	Diet (% GFLAX)*			Month**					*P*-value[2]		
FA (% of total FA)	0	10	SEM	June	July	Aug.	Sept.	SEM	T	M	T×M
4:0	5.30	5.24	0.071	5.61^{a}	5.25^{b}	5.17^{b}	5.05^{c}	0.060	0.59	<0.0001	0.95
6:0	2.52	2.22	0.045	2.42^{a}	2.42^{a}	2.31^{b}	2.34^{b}	0.035	0.0001	<0.0001	0.03
8:0	1.43	1.16	0.028	1.29^{b}	1.35^{a}	1.23^{c}	1.31^{ab}	0.024	<0.0001	0.0001	0.02
10:0	3.09	2.27	0.073	2.57^{b}	2.87^{a}	2.51^{b}	2.77^{a}	0.062	<0.0001	<0.0001	0.94
10:1	0.33	0.24	0.009	0.25^{c}	0.28^{b}	0.29^{ab}	0.31^{a}	0.009	<0.0001	<0.0001	0.71
11:0	0.04	0.03	0.002	0.02^{b}	0.04^{ab}	0.03^{b}	0.04^{a}	0.002	0.001	<0.0001	0.93
12:0	3.48	2.45	0.092	2.76^{b}	3.18^{a}	2.80^{b}	3.12^{a}	0.076	<0.0001	<0.0001	0.25
13:0	0.08	0.07	0.002	0.07^{b}	0.08^{a}	0.07^{b}	0.08^{a}	0.002	0.0002	<0.0001	0.77
14:0	11.29	8.62	0.222	9.38^{c}	10.27^{a}	9.82^{b}	10.34^{a}	0.182	<0.0001	<0.0001	0.06
iso 14:0	0.26	0.27	0.006	0.29^{a}	0.25^{c}	0.28^{ab}	0.26^{c}	0.007	0.23	0.0001	0.57
14:1	0.88	0.62	0.027	0.59^{c}	0.74^{b}	0.81^{a}	0.87^{a}	0.029	<0.0001	<0.0001	0.48
15:0	0.25	0.23	0.006	0.27^{a}	0.23^{c}	0.25^{b}	0.22^{c}	0.006	0.09	<0.0001	0.85
iso 15:0	0.31	0.27	0.012	0.31^{a}	0.28^{b,c}	0.30^{ab}	0.27^{c}	0.011	0.06	<0.01	0.78
anteiso 15:0	0.43	0.38	0.010	0.43^{a}	0.41^{b}	0.40^{b}	0.40^{b}	0.010	<0.01	0.001	0.82
16:0	0.43	0.41	0.011	0.44^{a}	0.42^{a,c}	0.43^{ab}	0.38^{c}	0.013	0.10	0.001	0.05
iso 16:0	0.13	0.11	0.004	0.11^{b}	0.11^{b}	0.13^{a}	0.12^{b}	0.005	0.02	0.013	0.57
16:1	0.28	0.22	0.010	0.27^{a}	0.26^{a,b}	0.24^{ac}	0.22^{c}	0.012	0.001	0.04	0.29
17:0	0.02	0.03	0.001	0.03^{a}	0.03^{b}	0.02^{b}	0.02^{b}	0.001	<0.0001	0.002	0.56
iso 17:0	12.05	16.40	0.364	16.52^{a}	13.75^{b}	13.65^{b}	12.98^{b}	0.439	<0.0001	<0.0001	0.31
anteiso 17:0	0.02	0.04	0.001	0.04^{a}	0.03^{b}	0.02^{b}	0.03^{b}	0.001	<0.0001	<0.0001	0.69
17:1	0.28	0.45	0.006	0.42^{a}	0.36^{b}	0.34^{b}	0.33^{c}	0.006	<0.0001	<0.0001	0.85
18:0	0.21	0.32	0.004	0.29^{a}	0.26^{b}	0.26^{b}	0.26^{b}	0.004	<0.0001	<0.0001	0.42
cis-9 18:1	16.51	21.90	0.562	19.53^{a,b}	18.09^{c}	20.01^{a}	19.20^{b}	0.425	<0.0001	<0.0001	0.54
$\sum$ *cis* 18:1[3]	17.17	22.92	0.574	20.38^{a,b}	18.92^{c}	20.84^{a}	20.04^{b}	0.434	<0.0001	<0.0001	0.57
trans-10 18:1	0.27	0.35	0.007	0.35^{a}	0.31^{b}	0.30^{bc}	0.28^{c}	0.007	<0.0001	<0.0001	0.02
trans-11 18:1	2.43	2.63	0.080	2.84^{a}	2.48^{b}	2.45^{b}	2.35^{b}	0.069	0.09	<0.0001	0.96
$\sum$ *trans* 18:1[4]	3.23	3.84	0.090	3.97^{a}	3.47^{b}	3.42^{b}	3.27^{c}	0.078	<0.001	<0.0001	0.99
cis-9, *trans*-11 CLA	0.92	0.95	0.026	0.89^{b}	0.89^{b}	1.00^{a}	0.97^{a}	0.023	0.45	<0.0001	0.65

Table 11. Milk fatty acid composition continued.

	Diet (% GFLAX)*			Month**					*P*-value[2]		
FA (% of total FA)	0	10	SEM	June	July	Aug.	Sept.	SEM	T	M	T×M
∑18:2, n-6	2.12	1.82	0.042	2.11^{a}	1.95^{b}	1.86^{c}	1.96^{b}	0.038	<0.0001	<0.0001	0.59
∑ 18:3, n-6	0.03	0.02	0.002	0.03^{a}	0.02^{a,b}	0.02^{a,b}	0.02^{b}	0.002	0.0001	0.13	0.21
∑18:3, n-3	0.62	1.16	0.030	0.87^{b}	0.89^{a,b}	0.89^{a,b}	0.91^{a}	0.024	<0.0001	0.10	0.14
20:0	0.18	0.19	0.003	0.20^{a}	0.18^{b}	0.18^{b}	0.18^{b}	0.003	0.01	<0.0001	0.39
20:2	0.04	0.03	0.001	0.03	0.03	0.03	0.03	0.001	<0.0001	0.58	0.01
20:3, n-6	0.09	0.06	0.004	0.07^{b}	0.08^{a,b}	0.07^{b}	0.08^{a}	0.003	<0.0001	0.01	0.61
20:4, n-6	0.08	0.06	0.003	0.07	0.07	0.07	0.07	0.002	<0.0001	0.59	0.02
20:5, n-3	0.04	0.05	0.001	0.05^{a}	0.04^{b}	0.04^{b}	0.04^{b}	0.002	0.03	0.0001	0.40
22:0	0.07	0.07	0.002	0.07^{a}	0.07^{b}	0.07^{b}	0.07^{b}	0.002	0.12	<0.0001	0.79
22:5, n-3	0.10	0.11	0.003	0.11	0.11	0.10	0.11	0.003	0.03	0.40	<0.01
24:0	0.05	0.07	0.002	0.06	0.06	0.06	0.06	0.002	<0.0001	0.38	<0.0001
∑ OBCFA[5]	1.70	1.52	0.042	1.72^{a}	1.57^{c}	1.66^{b}	1.50^{d}	0.033	0.01	<0.0001	0.03
∑SFA[6]	67.55	59.89	0.742	63.50^{b,c}	64.88^{a}	62.81^{c}	63.69^{b}	0.574	<0.0001	<0.0001	0.19
∑ n-6	2.33	1.96	0.047	2.28^{a}	2.13^{b}	2.03^{c}	2.13^{b}	0.041	<0.0001	<0.0001	0.64
∑ n-3	0.77	1.32	0.032	1.03	1.04	1.04	1.06	0.026	<0.0001	0.26	0.13
n-6/n-3	3.02	1.51	0.048	2.43^{a}	2.27^{b}	2.17^{c}	2.18^{b,c}	0.042	<0.0001	<0.0001	0.01
∑ MUFA[7]	22.63	28.40	0.562	25.98^{a,b}	24.30^{c}	26.31^{a}	25.48^{b}	0.426	<0.0001	<0.0001	0.16
∑ PUFA[8]	4.06	4.26	0.083	4.24^{a}	4.09^{b}	4.10^{b}	4.20^{a,b}	0.069	0.11	0.02	0.61
∑ 4:0 to 14:0	27.19	22.13	0.424	24.13^{b}	25.48^{a}	23.98^{b}	25.06^{a}	0.357	<0.0001	<0.0001	0.07
∑ < 16:0	28.09	22.94	0.419	24.94^{b}	26.36^{a}	24.82^{b}	25.93^{a}	0.356	<0.0001	<0.0001	0.07
∑ Unknown FAs	4.34	5.91	0.200	4.70^{b}	5.28^{a}	5.26^{a,b}	5.26^{a,b}	0.235	<0.0001	0.18	0.10

*n = 10, **n = 20.

[1]GFLAX; diets supplemented with ground flaxseed at 0 or 10% of diet DM.

[2] T = control vs. flaxseed, M = month, T×M = interaction of treatment with month.

[3]∑ *cis* 18:1; *cis*-9 18:1 + *cis*-11 18:1 + *cis*-12 18:1.

[4]∑ *trans* 18:1; *trans*-4 18:1 + *trans*-5 18:1 + *trans*-6-8 18:1 + *trans*-9 18:1 + *trans*-10 18:1 + *trans*-11 18:1.

[5]OBCFA; Odd-branched chain fatty acids.

[6]SFA; saturated fatty acids.

[7]MUFA; monounsaturated fatty acids.
[8]PUFA; polyunsaturated fatty acids.

Table 12. Received milk price, feed costs, and income over feed cost of cows fed 0 or 10% GFLAX[1] during the grazing season.

	Month			
Item	June	July	Aug.	Sept.
Milk, $/kg	0.75	0.72	0.74	0.75
Baleage, $/kg DM	0.23	0.22	0.22	0.20
Pasture, $/kg DM	0.19	0.18	0.18	0.17
Ground flaxseed, $/kg DM	1.32	1.36	1.36	1.35
Corn/soy mix[2], $/kg DM	0.92	0.88	0.89	0.88
Basal concentrate mix[3], $/kg DM	0.80	0.79	0.80	0.78
Liquid molasses, $/kg DM	0.84	0.84	0.84	0.84

	Diet (% GFLAX)	
	0	10
Total milk income, $/cow/d	13.41	13.07
Total feed cost, $/cow/d	7.84	8.47
TMR[4] cost, $/cow/d	6.79	7.33
Pasture cost, $/cow/d	1.06	1.14
IOFC[5], $/cow/d	5.53	4.61

[1]GFLAX; diets supplemented with ground flaxseed at 0 or 10% of diet DM.
[2]Corn/soy mix was top-dressed as the control treatment.
[3]Basal concentrate mix was included in the TMR for both 0 and 10% GFLAX diets.
[4]TMR is composed of baleage, basal concentrate mix, and top-dressed ground flaxseed or corn/soy treatments.
[5]IOFC = income over feed cost calculated by [total milk income ($/cow/d) – total feed cost ($/cow/d)].

APPENDIX A.

Institute for Animal Care and Use Committee Approval

University *of* New Hampshire

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

23-Jun-2011

Brito, Andre Fonseca De
Biological Sciences, Dairy T & R Ctr
Durham, NH 03824

IACUC #: 110605
Project: Feeding Whole Flaxseed to Organic Dairy Cows
Category: C
Approval Date: 23-Jun-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:

1. Amanda Craig needs to complete the occupational health program for animal handlers prior to handling any vertebrate animals.
2. In future applications, the information in Section III, B needs to be specific to the study proposed, and not so general in nature.
3. Before any portion of the study is initiated outside New Hampshire, where applicable the researcher needs to submit to the UNH IACUC for the file a copy of the IACUC approval letter from the applicable university.

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,
Jessica Bolker

Jessica A. Bolker, Ph.D.
Chair

cc: File
Whitehouse, Nancy

University *of* New Hampshire

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

11-Jun-2013

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

IACUC #: 130505
Project: Impact of Ground Flaxseed on Carbon Emissions in Lactating Organic Cows During the Grazing Season
Category: C
Approval Date: 23-May-2013

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, Ph.D.
Chair

cc: File