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EFFECTS OF FLAXSEED-BASED FEED SUPPLEMENT ON PRODUCTION PERFORMANCE, ENERGY UTILIZATION, MILK FATTY ACID PROFILE, AND ENTERIC METHANE EMISSIONS IN JERSEY COWS GRAZING MIXED GRASS-LEGUME PASTURE

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ABSTRACT

EFFECTS OF FLAXSEED-BASED FEED SUPPLEMENT ON PRODUCTION PERFORMANCE, ENERGY UTILIZATION, MILK FATTY ACID PROFILE, AND ENTERIC METHANE EMISSIONS IN JERSEY COWS GRAZING MIXED GRASS-LEGUME PASTURE

by
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This study investigated the effect of an extruded flaxseed-based feed supplement 'LinPRO-R (**LNPR**)' on milk production and composition, milk FA profile, nutrient digestibility, ruminal metabolism, purine derivatives (**PD**) excretion, and enteric methane ($\mathbf{CH_4}$) emissions in grazing dairy cows during the summer season. Eighteen multiparous and 2 primiparous mid-lactating organic Jersey cows (128 ± 52 DIM) were used in a randomized complete block design. cows grazed mixed grass-legume pasture (*Dactylis glomerata* L., *Trifolium repens* L., *Trifolium pratense* L., *Lolium perenne* L., *Phleum pratense* L.) overnight (herbage allowance = 15 kg of DM/cow/day) following a strip grazing method and fed partial total-mixed ration (**pTMR**) in the barn during the day. The pTMR was formulated to contain (DM basis) 37.5% mixed, mostly legume baleage and 62.5% of a soybean meal/ground corn-based concentrate mash. Cows within pairs were randomly assigned to 1 of 2 diets: (1) pasture plus pTMR (control diet= **CTRL**) or (2) pasture, pTMR, and 6% LinPRO-R (LinPRO-R diet = **LIN**). Ground corn and soybean (extruded and roasted) were replaced with LinPro-R in the LIN diet. Diets were formulated to be isonitrogenous and to yield a 60:40 forage to concentrate ratio. Pasture averaged 17.5% CP and 53% NDF, and pTMR 9.7% CP and 15% NDF. The experiment lasted 12 wk with 2 wk for a covariate period followed by 3 sampling periods during wk 4, 7, and 10. Individual herbage intake was estimated using Cr_2O_3 and the in vitro dry matter digestibility (IVDMD) of the feeds. Two GreenFeed units were used to measure gaseous

emissions throughout the study. Cows on LIN diet were observed to have a lower herbage intake (5.95 vs. 7.39 kg/d; $P < 0.01$) compared with CTRL, whereas the pTMR dry matter intake (**DMI**) was similar (mean = 14.7 kg/d) between the diets. Intake of OM (21.2 vs 20.3 kg/d), CP (3.82 vs 3.52 kg/d), NDF (7.55 vs 6.83 kg/d), and ADF (5.21 vs 4.80 kg/d) were greater ($P \leq 0.05$) in CTRL compared to LIN. Contrarily, apparent total tract digestibility of DM (70.5 vs 69.5%), OM (71.5 vs 70.4%), and CP (65.7 vs 64.8%) were greater in LIN compared to CTRL whereas ADF and NDF digestibility did not differ. Treatments had no effect on milk yield (mean = 27 kg/d), and milk components. However, milk urea nitrogen (**MUN**) concentration was lower ($P < 0.001$) in LIN (8.38 mg/dL) than CTRL (11.0 mg/dL). No treatment effects were observed for total VFA concentration (mean = 89.8 m*M*), and the molar proportions of acetate, propionate, butyrate, and the acetate-to-propionate ratio (mean = 4.6). Similarly, production of CO_2 (mean = 10.9 kg/d), enteric CH_4 (mean = 351 g/d), CH_4 yield (mean = 15.5 g/kg of DMI) and CH_4 intensity (mean = 11.3 g/kg of ECM) did not differ with feeding CTRL vs. LIN. Most of the milk saturated fatty acids (**SFA**), Σ odd-chain, Σ branched -chain SFA, Σ<16C, Σ16C, and Σn-6 FA increased ($P<0.01$) in CTRL compared to LIN. In contrast, majority of the Σ18C FA, unsaturated fatty acids (**UFA**), *trans*-11 18:1, *cis*-9, *cis*-12, *cis*-15 18:3, *cis*-9, *trans*-11 18:2, and Σn-3 FA increased ($P<0.01$) in cows fed LIN diet than those fed CTRL. The Σn-6/n-3 ratio decreased with feeding LIN versus CTRL. In summary, LinPRO-R fed at 6% diet DM did not affect production performance and enteric CH_4 emissions in grazing dairy cows but increased Σn-3 FA in milk. Thus, the profitability of LIN inclusion in the pasture based-dairy system will be contingent upon the cost involved and the industry acceptance of premium n-3 enriched milk.

Key words: Extruded flaxseed, pasture, milk yield, α-linolenic acid, greenhouse gas

CHAPTER I. REVIEW OF LITERATURE

Introduction

Organic dairy is one of the fastest-growing US organic industries. However, organic dairy farmers are facing multiple challenges, including high grain costs, legume persistence, and forage quality, which can threaten the economic sustainability of their family enterprises (McBride and Greene, 2009). To reduce feed costs and keep organic certification, organic dairy farmers generally feed high-forage diets and rely heavily on grazed herbage during the summer months (Marston et al., 2011). It is well established that pasture-based diets lack energy, and there is a need to supplement energy sources in the diets of grazing dairy cows to mitigate any potential production losses (Bargo et al., 2002a; Hafla et al., 2016). Therefore, supplementing oilseeds to high-yielding dairy cows to enhance the energy density of their diet has become a common strategy among many dairy operations. Currently, there is a growing interest in incorporating flaxseed to increase the energy density of the diet, which increase milk yield and components, omega-3 (n-3) fatty acids (**FA**) concentration, especially α-linolenic acid (**ALA),** and reduce enteric CH_4 emissions in dairy cows. Flaxseed has been fed to dairy cows in many different forms for instance, ground flaxseed, extruded flaxseed, micronized flaxseed, flaxseed hulls, and flaxseed oil. Numerous studies have investigated the effects of ground flaxseed (Da Silva et al., 2007; Petit and Cortes, 2010; Petit, 2010; Resende et al., 2015; Isenberg et al., 2019) and extruded flaxseed (Gonthier et al., 2004; Ferlay et al., 2013; Neveu et al., 2013, 2014; Lerch et al., 2014a, b, c) on production performance, nutrient digestibility, and milk FA profile in dairy cows. Feeding extruded flaxseed has also been shown to reduce enteric CH_4 emissions (Martin et al., 2008; Martin et al., 2016).

The LinPRO-R (O&T Farms Ltd., Regina, SK, Canada) is an extruded flaxseed-based feed supplement that is prepared by dry extrusion process. Previous studies with feeding LinPRO-R to the dairy cows in confinement have been shown to increase milk yield and components and n-3 FA concentration in milk (Moats et al., 2018; Swanepoel and Robinson, 2019a, b) but no effects on enteric CH_4 emissions (Judy et al., 2019). However, studies on the effects of extruded flaxseed-based supplement in grazing dairy cows are limited. This literature review aims to discuss the prospects and challenges of organic dairy, different supplementation strategies for grazing dairy cows, lipid metabolism and ruminal biohydrogenation and the synthesis of milk FA. Also, the goal of this literature review is to discuss the effects of different forms of flaxseed on energy utilization, production performance, nutrient digestibility, milk FA profile, and gaseous emissions in dairy cows.

Organic Dairy Farming

Organic dairying is defined by the United States Department of Agriculture (USDA) as a "method of milk production under the act of organic production that integrates cultural, biological and mechanical practices which avoid the use of synthetic fertilizer, sewage sludge, irradiation, pesticides, and genetic modified organisms (GMOs)" in addition to "managing livestock to promote and enhance biodiversity, biological cycles, and better utilization of natural resources" (USDA-NOP, 2022). The term "organic" is used throughout this thesis in accordance with these specifications.

Some other USDA organic guidelines are outlined by Coffey and Baier (2012). The cows should have a minimum of 120 days of access to pasture during the grazing season and at least 30% of their daily dry matter intake (**DMI**) should come from pasture (USDA-AMS, 2010). Additionally, all the feed ingredients fed to the cows must be certified organic and grown

without the use of GMO. Furthermore, organic cow's diet is not allowed to contain urea, manure, and mammalian or poultry slaughter by products (USDA-NOP, 2022). If any cow gets sick, and requires antibiotics, producers are recommended to treat the animals with antibiotics; however, treated animals will no longer maintain organic status and the milk from those cows cannot be sold as organic. In any case, the use of growth promoting drugs including hormones is prohibited in the organic dairy production. Producers also must ensure animal welfare and comfortable living conditions, which means the cows should not be in a confinement system such as tie stalls. Whereas the use vaccines are permitted, the routine use of antiparasitic drugs are prohibited (USDA-NOP, 2022).

Prospects and Challenges of Organic Dairy Farming

Organic dairying has been one of the fastest growing segments in US organic agriculture in the past decades. Many conventional dairy producers have shifted to organic production due to the exceeding demand than supply (McCrory et al., 2001), and a constant milk price throughout the year (Dalton et al., 2008). A comparative analysis between Vermont and Maine found that the number of organic dairy farms in Vermont was only 2 in 1993, and increased to 200 in 2008 (Dalton et al., 2008), while Maine went to 25 farms in 1993 to 60 certified organic dairy farms today (USDA-NASS, 2021). In 2011, there were only 12 certified organic dairy farms in New Hampshire (USDA-NASS, 2011), but this number has increased to 20 in 2019 (USDA-NASS, 2019). In fact, the Northeast was the home of more than 80% of the US organic dairies but these cows are less productive than that of west (McBride and Greene, 2009). In 2011, the US had a total of 1,848 organic dairies that produced 1.39 billion tons of milk with annual sales reaching $0.76 billion (USDA-NASS, 2011). More recently, the USDA-NASS (2019) survey showed that

the number of organic dairy farms increased to 3,134 that produced 2.56 billion tons of milk worth of $1.58 billion.

Using an annual survey from the Agricultural Resource Management (ARM), McBride and Greene (2009) reported that organic dairy cows increased by 25 percent in the US going from 3,8000 to 8,6000 between 2000 and 2005. It was also reported that 45% of the organic dairies milk less than 50 cows, and 87% of the total organic milk originated from farms that raise below 100 cows (McBride and Greene., 2009). A 10-year longitudinal data (2006-2016) from the Vermont organic dairies showed that, on average, each farm had 66 cows (Walsh et al., 2020). Overall, Northeast organic dairies had an average of 53 cows, while upper Midwest and West had an average of 64 and 381 cows, respectively (McBride and Greene, 2009).

Indeed, the organic dairy sector has made a significant progress in the last decades; however, many producers nowadays are struggling to stay in business due to the limited land, extreme weather, volatile milk prices, increased feed costs, and production quotas (McBride and Greene, 2009; Walsh et al., 2020, Hardie et al., 2014; Hennesy et al., 2020). A survey from USDA-NASS (2012) revealed that the feed cost comprises 50% of total cost in organic dairy operations. Additionally, the scarcity of grazing land and quality pasture is adding other constraints and putting the organic enterprise at risk. Besides, the requirement for 100% organically certified feed despite high grain costs creates a significant challenge for organic dairy producers explaining why most producers tend to rely heavily on pasture. However, pasture-based diet lacks energy and the aftermath is production losses during the grazing season (Bargo et al., 2002a). Therefore, the overall organic farm profitability does not meet the expected standard compared to conventional cows (Walsh et al., 2020). McBride and Greene (2009) also reported that the rigorous certification process to be organic is another limitation.

As a result of increased feed costs and unstable milk prices, the number of organic dairies in the US has started to decline in recent years. Data from USDA-NASS (2021) showed a 19% reduction of organic dairies nationwide from 3,134 in 2019 to 2,528 farms in 2021.

Ruminal Lipid Metabolism and Biohydrogenation

High-producing dairy cows typically consume a diet that contains approximately 6% lipids (>90% FA), with grass and grains each contributing 3% and the remaining amount coming from supplementary fat (Bionaz et al., 2020). Lipid in the diet plays a vital role in maintaining reproductive health and metabolism. However, significant improvements in the understanding of lipid digestion and metabolism in ruminant have been done between 1950 to 1980. Therefore, recent focus has been shifted to dietary manipulation to increase the nutritional quality of ruminant food products, for example, enriched milk with n-3 FA especially ALA.

Figure 1 illustrates the common types of fat found in different feed ingredients and their metabolism within the rumen. There are 3 different types of lipids primarily entering into the rumen: triglyceride (TG), phospholipids, and galactolipids. The fate of the complex lipid is FA and glycerol by the action of microbial lipases which break down the ester bonds of the dietary lipids (Jenkins 1993; Jenkins et al., 2010). The microbial activity of the hydrolysis of different lipids is highly specific, and the rate of hydrolysis differs significantly depending on the action of the microorganisms (Buccioni et al., 2012). After lipolysis, the unsaturated FA undergoes biohydrogenation. Biohydrogenation refers to the process through which double bonds in unsaturated FA are converted into single bonds via isomerization (Jenkins and McGuire, 2006). Thus, there is a noticeable difference observed between the FA that enter to the rumen, are predominantly polyunsaturated FA (PUFA) derived from the animal's diet (Drackley, 2007), and

those that exit the rumen, which are mainly saturated FA due to the biohydrogenation process (Chilliard et al., 2000; Lock and Bauman, 2004).

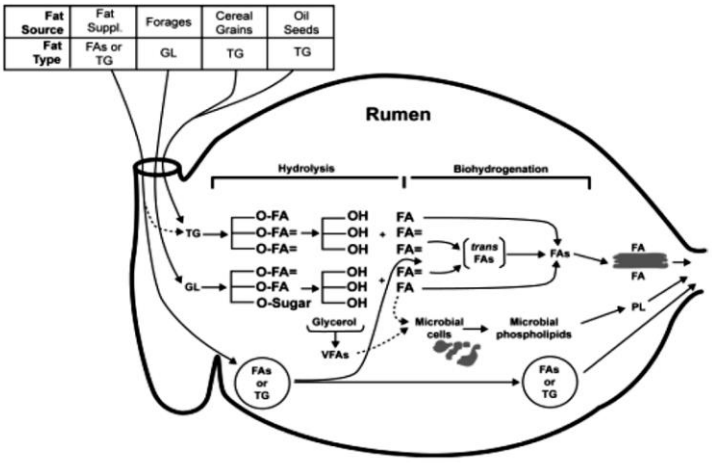

Fig. 1. Common fat types found in different feed ingredients and their metabolism (TG = triacylglycerides, GL= glycolipids, FA= fatty acids; Bauman and Lock, 2006).

(A)

cis-9, *cis*-12, *cis*-15-18:3

↓

cis-9, *trans*-11, *cis*-15-18:3

↓

trans-11, *cis*-15-18:2

↙ ↘

cis-15 and *trans*-15-18:1 *trans*-11-18:1

↘ ↙

18:0

(B)

cis-9, *cis*-12-18:2

↓

cis-9, *trans*-11-18:2

↓

trans-11-18:1

↓

18:0

(C)

cis-9-18:1 → *trans*-18:1

↘ ↙

18:0

Fig. 2. Biohydrogenation pathways of (A) α-linolenic, (B) linoleic, and (C) oleic acids (Harfoot and Hazlewood;1988).

Biohydrogenation pathways are outlined by Harfoot and Hazlewood, (1988). Figure 2 illustrates the conversion of ALA, *cis*-9, *cis*-12 18:2 (**CLA**) and *cis*-9 18:1 (oleic acid) through biohydrogenation process. The first step of biohydrogenation is isomerization where the conversion of *cis*-12 to *trans*-11 18:2 occurs. The next step is the hydrogenation reaction where the double bond is replaced by single bond that produce *trans*-11 structure from *cis*-9 (Fig. 2: B). In the last step, the *trans*-11 double bond is hydrogenated again resulting in 18:0 FA. The hydrogenation of CLA and ALA can range anywhere from 70 to 95% and 85 to 100%, respectively, depending on the diet, the type and nature of fat supplementation, and ruminal pH (Jenkins et al., 2008). Therefore, as the supplies of unsaturated FA in the diet increases, the rate of biohydrogenation increases too (Bauman and Lock, 2006).

To evaluate the rate and extent of biohydrogenation, it is important to understand the interrelationships among the diet, the type and amount of dietary lipids, ruminal fermentation, and the synthesis of milk fat in mammary gland. For example, the degree of biohydrogenation largely depends on the forage: concentrate ratio (Dewhurst et al., 2006). When the forage: concentrate ratio increased from 60:40 to 25:75 in diet containing corn silage and alfalfa haylage, biohydrogenation of *trans* 18:1 FA decreased (Kalscheur et al., 1997). Ruminal biohydrogenation of conserved forages also depends on the type of forages fed to the cows. It has been observed a greater transfer efficiency of CLA (9.0 vs. 4.5%) in the diet containing red clover silage than grass silage which is possibly due to the higher content of polyunsaturated FA in legumes and the polyphenol oxidase activity in red cover (Dewhurst et al., 2006). Likewise,

lower biohydrogenation of CLA was observed in organic certified cows than conventional which is likely related to the higher amount of legume forage (Couvreur et al., 2006).

Studies have also investigated the effects of different oilseeds on the dynamics of ruminal biohydrogenation of long chain fatty acids (**LCFA**) both in-vitro and in-vivo. Hoffman et al. (2015) evaluated ruminal biohydrogenation of LCFA using 4 different oilseeds (flaxseed, soya beans, sunflower seeds, and rapeseed) in-vitro. It was observed that 40-60% FA double bonds disappeared in all the oilseeds after 24 h of incubation. Also, a greater concentration of biohydrogenation intermediates (*cis*-9, *trans*-11 18:2 and *trans*-11 18:1) were found in both flaxseed and sunflower seeds compared to soybeans and rapeseed which was likely due to the higher concentration of ALA and CLA in both oilseeds.

Ferlay et al. (2013) conducted 2 experiments in which incremental amounts of extruded flaxseed (0, 5, 10, and 15% of diet DM) was fed either with hay (experiment 1) or corn silage-based diet (experiment 2). Feeding extruded flaxseed increased *trans* isomers of 18:1 in milk fat linearly, thereby the transfer efficiency of ALA and CLA in milk also decreased linearly when flaxseed was fed either with hay or corn-silage based diet.

Milk Fatty Acid Synthesis

One of the largest portions of milk fat is saturated FA (70%). The second largest part is monounsaturated FA (25%) with the least being polyunsaturated FA (5%) (Grummer et al., 1990). Triglycerides make up between 96 and 99% of the lipids in milk fat, while phospholipids and sterols make up less than 1% and 0.5% of the fat, respectively (Patton and Jensen 1976; Timmen and Patton, 1988). Furthermore, there are more than 400 distinct FA found in ruminant

milk fat, with the most abundant being saturated FA as mentioned earlier, with FA varying in chain lengths from 4 to 18 carbon atoms (Shingfield et al., 2013).

The composition of milk fat produced by ruminants is unlike that of any other mammals because of the wide variety of FA that it contains. This diversity is due to the *de novo* synthesis of FA in the mammary gland and the impact of ruminal biohydrogenation on dietary unsaturated FA (Palmquist, 2006). However, the question of whether milk fat comes entirely from the diet of the animal or is produced by the animal itself was an early point of contention among researchers (Jordan and Jenter, 1987). Several studies have been done in the past 25 years to understand the nutritional and molecular mechanisms of milk FA synthesis (Dills 1984; Grummer 1990; Chilliard et al., 2000; Jenkins and McGuire, 2006; Palmquist 2006; Jenkins et al., 2010).

Synthesis of milk fat can be categorized in 2 different ways: 1) synthesis of FA in the mammary gland or *de novo* and 2) uptake of performed FA from the diet or non-esterified fatty acid (**NEFA**) from the body stores. The requirements for the synthesis of FA in the mammary gland or *de novo* are carbon sources and the reducing equivalents NADPH + H^+. ß-hydroxybutyrate and acetate are the primary sources of carbon in ruminant animals derived from the fiber fermentation in the rumen while glucose and acetate are the sources of reducing equivalents (Bauman and Davis 1974; Lock and Bauman, 2004).

De novo fatty acid synthesis appears to be responsible for almost all the 4:0 to 14:0 fatty acids and for one half of the 16:0 fatty acid found in milk (Grummer 1990). Figure 3 depicts the mechanism by which the FAS complex facilitates the enzymatic reaction for *de* novo FA synthesis. The starting point of *de novo* synthesis of FA is to use the acetate from blood (+CoA +ATP) which is then converted to AMP and acetyl-CoA by cytosolic acetyl-CoA synthase. An alternative pathway of glucose oxidation is pentose phosphate pathway (**PPP**) that utilizes

glucose and provides reducing equivalents NADPH + H^+ for FA synthesis and glycerol-3-phosphate for FA esterification. Wood et al. (1965) first reported that 30% of glucose in ruminant animals was metabolized by PPP, 10% by Meyerhof pathway, and the rest 60% glucose was converted to lactose. After NADPH + H^+ is provided by the PPP, acetyl-CoA is used as a primer of malonyl-CoA in a reaction catalyzed by acetyl-CoA carboxylase. The next step is the elongation process catalyzed by the fatty acid synthase. The last step is the release of FA from fatty acid synthase complex. The FA is then sent to the endoplasmic reticulum, where it is esterified with glycerol and incorporated into a milk fat droplet as triglycerides.

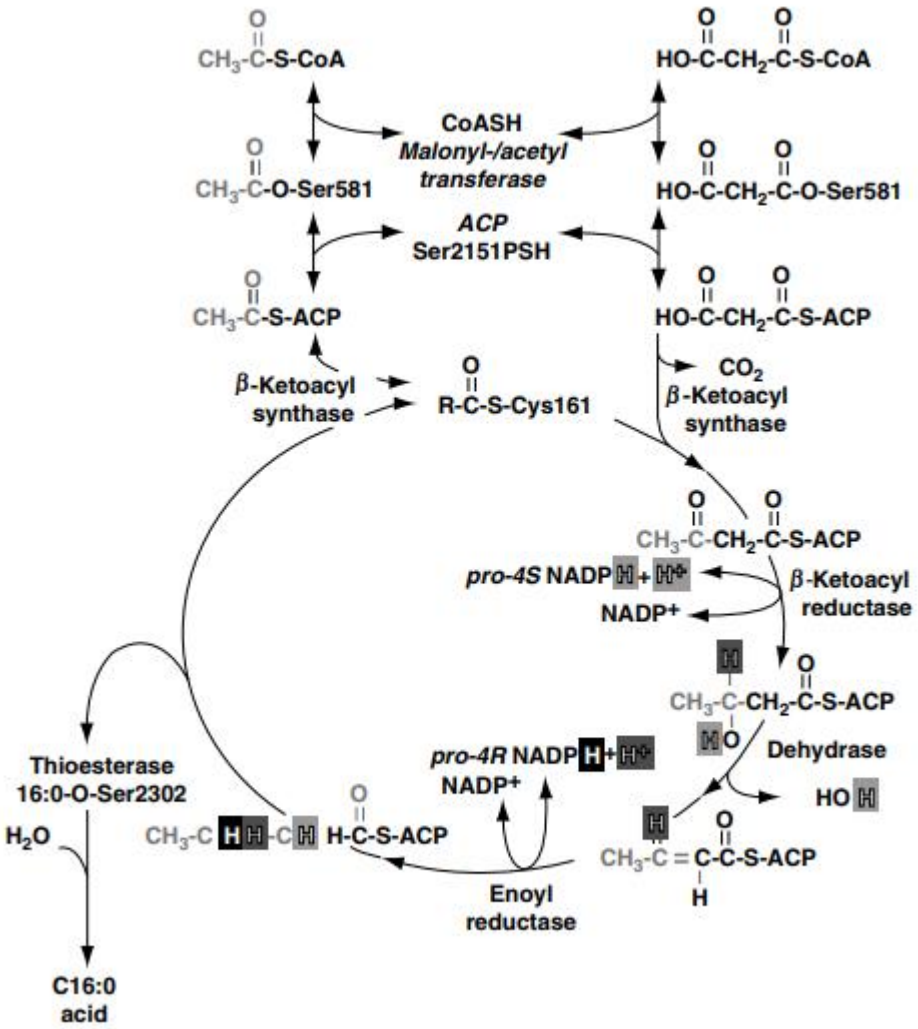

Fig. 3. Biosynthesis of FA *de novo* catalyzed by fatty acid synthase (FAS) complex. (Smith et al., 2003).

The type of milk FA originated from the dietary and microbial lipid absorption by the intestine as well as the mobilization of stored body fat are 16:0 FA (partially) and all other long chain FA >16:0 (Bauman and Griinari, 2003; Lock and Bauman, 2004). The FA that arrives in the small intestine are no different than those leaving the rumen because there are no changes to those FA in the omasum or abomasum (Moore and Christie, 1984). Therefore, the mammary gland uptake of FA from the blood is basically either triglyceride rich lipoprotein or NEFA.

The plasma concentration of NEFA largely depends on the extent of fat mobilization which is again related to the energy status of the animal (Chilliard et al., 1984). Non-esterified fatty acids (NEFA) usually contribute 10% to the overall milk FA synthesis; however, when the animals are in negative energy balance, this contribution increases proportionally to the extent of the energy deficit (Ven Knegsel et al., 2006). Moreover, the enzyme lipoprotein lipase (**LPL**) determines the ability of mammary gland to uptake triglyceride FA from bloodstream (Chilliard et al., 2000). There is a positive correlation between the plasma concentration of triglyceride FA and the uptake of mammary tissues. In opposite to other body tissues, mammary tissue is unable to convert 16:0 to 18:0 FA using the elongation process but what they are capable of is called "desaturation" where the mammary tissue utilizes 18:0 and converts those to *cis-9* 18:1 using delta-9 desaturase enzyme (Kinsella and Stearyl, 1972). Approximately 40% 18:0 FA is converted through this mechanism and thus contributing more than 50% 18:0 in milk FA (Enjalbert et al., 1998). Furthermore, another *trans* FA, vaccenic acid (*trans-11* 18:1) that is formed in the rumen via biohydrogenation, is converted to rumenic acid (*cis-9, trans-11* 18:2) which is one of the major CLA isomers (Griinari and Bauman, 1999).

The glycerol-3 phosphate pathways are considered as one of the primary routes for the formation of triglycerides in ruminant mammary tissue. Two molecules that are needed for the esterification are acyl-coA ester and glycerol-3 phosphate where glycolysis and PPP are the primary sources of glycerol-3 phosphate (Dils, 1984).

LinPRO-R Background

LinPRO-R is a commercial dry extruded organic-certified flaxseed-based product marketed by O&T Farms (O&T Farms Ltd., Regina, SK, Canada) that is designed to supplement dairy cows. This product is comprised of flaxseed, alfalfa, and field peas which are excellent sources of polyunsaturated fatty acids (**PUFA**) including n-3 fatty acids (FA). Ingredients composition of LinPRO-R is listed in Table 1. The company claims that LinPRO-R can be used as a fat supplement to support the energy density of the dairy cows' diet, thus improving milk production, reproductive performance, and overall health. Some additional benefits may include reducing inflammation, strengthening immune system, and enhancing milk quality with n-3 FA (Swanepoel and Robinson, 2019a; b)

Several processing methods have been applied to flaxseed in the past including grinding, extrusion, roasting, and alkaline and acid treatments to increase the nutritional value and digestibility of flaxseed for high yielding dairy cows (Mustafa et al., 2003a; Mustafa et al., 2003b). Processing methods can have a large impact on animal performance and milk quality, particularly on milk production, and milk FA profile depending on the stage of lactation, forage-to-concentrate ratio, the type of forages used in the diet, pasture quality, and environmental condition. According to O&T Farms, a dry extrusion process is used to prepare LinPRO-R to denature the outer protein matrix of flaxseed, hence protecting fat from extensive ruminal biohydrogenation (Swanepoel and Robinson, 2019a). Therefore, LinPRO-R can be a tool for

dairy producers who are seeking a product for optimizing nutrient utilization and boost production of their herds.

Milk Production on Pasture versus TMR Diets

High quality pasture is one of the key components for maximizing profitability and resilience in organic dairy operations due to the USDA pasture regulation (USDA-AMS, 2010). However, farmer's ability to properly implement forage-based diets is hindered by issues related to legume persistence and abundance, lower herbage intake, unpredictable herbage growth and nutritive value, and proper energy:protein ratio in forages (Brito et al. 2008, 2009; da Silva et al. 2013, 2014; Wilkinson et al., 2018). Hafla et al. (2016) conducted a study across the Northeast region of the US in which pasture samples were collected from 14 organic dairy farms during the grazing season and found that the most limiting dietary component was energy. In fact, 86% and 21% of pastures failed to meet the minimum net energy of lactation (**NE_L**) and crude protein (**CP**) recommendations for Jersey cows, respectively. The authors also reported that grasses made up 67% of the pastures, while legumes contributed only 26%. It should be noted that climate, soil fertility, herd management, and grazing strategies all interact to affect the longevity and maintenance of the legume composition of a pasture, making it more than just an agronomic management issue (Ledgard and Steele, 1992).

Kolver and Muller (1997) compared total mixed rations (**TMR**) versus pasture feeding using 16 dairy cows. At the start of experiment in wk 1 and 2, the grazing cows received 50% and 25% TMR of their required DMI, respectively. In wk 3 and 4, grazing cows were solely on pasture and fed only trace minerals and water. The TMR cows were fed TMR in confinement. The study was divided into 2 portions: transition period and intake period. During the intake period, there was 19% drop in DMI for grazing cows and they produced less milk (29.6 vs 44.1

kg/d) with 2.61 vs 2.80% milk protein compared to the TMR counterparts. Milk fat yield was also reduced in grazing cows compared to TMR group.

A comparison by Knaus (2014) on pasture versus indoor feeding of dairy cows summarized that the larger the portion of pasture in the daily ration, the greater the cows' constraints in daily DMI, and the lower their potential for milk synthesis. This means that maximizing milk performance per cow is incompatible with a pasture-based feeding scheme. Therefore, DMI of pasture is one of the major contributing factors to the milk production of grazing dairy cows regardless of any grazing management used such as rotational or strip grazing that is driven by pasture allowance (Moate et al., 1999).

A meta-analysis by Perez-Prieto and Delagarde (2013) that included 97 papers on pasture allowance revealed that yield of milk and milk components increased with the increasing level of pasture allowance. This is consistent with the findings from a review by Kolver (2003) who recommended that dairy producers must be supplemented additional metabolizable energy (**ME**) or removing the constraints of *ad libitum* herbage intake by providing good quality pasture with sufficient pasture allowance to maintain milk production above 30 kg/d. Furthermore, providing cows with access to pasture for 6 h per day while feeding TMR in confinement did not improve intake or milk performance for cows producing around 45 kg of milk/d where pasture was contributing only 8% of the DMI (Atkins et al., 2020). Kennedy et al. (2005) concluded that a slightly higher production performance is attainable for spring-calving early lactating dairy cows if grazed high quality forage than those in confinement feeding systems with higher level of concentrates and silages.

Supplementation is one of the approaches to mitigate production losses in grazing dairy cows. Scharen et al. (2016) reported that transition from TMR to pasture decreased the molar

proportion of acetate, and increased butyrate throughout the experiment which perhaps due to the increased intake of fermentable carbohydrates. However, following the behavioral and metabolic adjustments from the TMR to pasture, no detrimental effects on rumen fermentation, and health were observed. The authors also reported that the TMR DMI started to decline as soon as the cows were moved to the pasture whereas the milk yield of pasture cows was higher than the confined group. Fontaneli et al. (2005) observed a similar effect on DMI, but the milk yield was greater in confined cows compared to those grazed pasture. However, this study did not find any differences in the overall profitability between these two-feeding systems.

In summary, existing research indicates that grazing dairy cows relying heavily on pasture are unable to meet the nutritional requirements, leading to production losses which is a challenge for the dairy producers to maximize farm profitability.

Performance of Grazing Dairy Cows with Different Supplementation Strategies

Pasture is considered as the cheapest source of nutrients for grazing dairy cows, and organic dairy producers rely heavily on grazed herbage for their cows during the grazing season to reduce feed costs as explained earlier (Clark and Kanneganti, 1998; Peyraud and Delaby, 2001). However, supplementation of grazed herbage is often necessary to maintain or improve the production performance considering the limitation between nutrient intake and requirements of the cows. Indeed, the constraints are multifactorial when it comes to the production inefficiency of grazing dairy cows. Therefore, there has been a growing emphasis on feed supplementation for grazing dairy cows to adequately fulfil their nutritional requirements.

Synchronization among the daily herbage allowance, type of grasses and legumes on pasture and nature of supplementation with partial total mixed ration (pTMR) is required to

maintain the milk production during grazing season. Indeed, it is very crucial to balance the energy: protein ratio in the diet of grazing dairy cows by providing different concentrate supplements. Therefore, providing supplementation has emerged as an important area of research for grazing dairy cows. Studies have investigated different supplementation strategies such as barley (Adams et al. 1995), corn (Bargo et al., 2002a), beet pulp and corn gluten meal (Kibon and Holmes, 1987), wheat, citrus pulp, and soybean meal (Sayers, 1999). Some other studies also investigated fat rich supplement such as sunflower seeds and oil (Gagliostro et al., 2017), sunflower meal and Calcium salts of FA (Schroeder et al., 2003), whole cottonseed and tallow (Adams et al., 1995), and hydrogenated fish fat (Gallardo et al., 2001).

Most of the studies conducted on starch or fat rich supplements reported a greater milk yield and components without any adverse effect on metabolism and health in cows. For example, Gagliostro et al. (2017) fed sunflower oil and sunflower seed with or without fish oil to grazing dairy cows in a 4 × 4 Latin square study with 2 × 2 factorial arrangement of treatments. Results from this study indicated that milk yield, fat-corrected milk, and milk protein concentration were greater with sunflower oil compared to the diet with sunflower seed. However, milk CLA and ALA concentrations in milk fat increased in cows fed fish oil.

Schroeder et al. (2004) reviewed 18 experiments to observe the effect of fat supplements in grazing dairy cows at different stages of lactation. The results indicated that feeding saturated FA to mid-lactation cows increased milk production and milk fat concentration, but unsaturated FA decreased milk fat concentration by 8%. The authors summarized that fat supplementation with high quality pasture usually increases milk production in grazing dairy cows, but the effects on milk composition is largely influenced by the extent of saturation of fat supplement.

Similar to what was found with supplemental fat, many studies also showed improved milk production when grazing cows were fed corn-based supplement with different pasture allowances. Bargo et al. (2002b) investigated high (40 kg DM/d) and low (25 kg DM/d) pasture allowance with or without supplementation of concentrates (dry shelled corn and wheat midds based concentrate;1 kg DM/4 kg of milk). Milk yield and 3.5% fat corrected milk (**FCM**), and true protein yield were greater with feeding supplements than those unsupplemented. Similarly, cows fed supplements had greater total DMI, but the herbage DMI decreased which was likely due to the substitution effects of supplements. Even though that milk fat% was reduced in supplemental group, yield of fat was greater because of higher milk yield. Additionally, the total volatile fatty acids (**VFA**) concentration increased in supplemental group, whereas plasma NEFA was reduced. The authors did not observe any changes in body weight (**BW**) and body condition score (**BCS**) among the treatments. Similarly, Tozer et al. (2004) compared 4 diets with high and low pasture allowance with or without shelled corn-based supplements. It was concluded that regardless of pasture allowances, the cows fed supplements produced more milk with a greater feed efficiency, yield of milk fat and true protein compared to unsupplemented cows. The difference in milk yield and composition was because of higher DMI in cows fed supplements. However, the income over feed cost was greater when supplements were fed with low pasture allowances compared to other diets. Also, the lowest economic return was observed when the cows were fed high pasture allowance with no supplementation. These findings are consistent with Soder et al. (2001) who evaluated 4 levels of different concentrates in grazing dairy cows and found that the farm profitability increased when added concentrates to grazing cow diets.

Studies also compared feeding corn-based concentrate with molasses or ca-salts of FA supplements. Results from a comparison of ground corn and liquid molasses in grazing cattle

diet reported a greater supplemental DMI for cows fed liquid molasses compared to those fed ground corn (Brito et al., 2017). There was no effect on milk production and yield and concentration of milk components, and plasma amino acids (**AA**) between the treatments. The authors suggested that it is possible to replace ground corn entirely with liquid molasses without affecting the production performance of dairy cows during the grazing season. Schroeder et al. (2003) evaluated 3 diets: TMR, pasture plus corn-based concentrate, and pasture plus corn-based concentrate with Ca salts of unsaturated FA in grazing dairy cows. No differences were observed in milk production, DMI, milk yield, and ruminal pH and total VFA concentrations among treatments. However, TMR cows produced more fat-corrected milk than pasture cows (19.5 vs. 16.1 kg/d), which was due to the higher milk fat concentration (3.91 vs. 2.56%) compared to pasture cows. Additionally, milk protein concentration was higher in TMR diet compared with the other 2 diets. However, the authors noted that the milk concentration of CLA was highest when the cows were fed the pasture-based diets. The CLA in milk is originated from 2 sources; 1) microbial biohydrogenation of dietary 18:2 FA which produce approximately 25% CLA, 2) desaturation process of vaccenic acid by mammary Δ9-desaturase which results about 75% of CLA in milk (Griinari and Bauman, 1999). It has been suggested that dietary supplementation of unsaturated long-chain FA in the diet of grazing dairy cows may potentially augment the positive effects of herbage intake on milk FA profile (Lawless et al., 1998).

It is well established that the energy is the most limiting factor in grazing dairy cows' diet, but adequate protein in the diet is also important. Ayers et al. (2021) evaluated 2 different levels of crude protein (CP) (Low:14.8% vs. High:19.3%) in grazing cows' diet in 6 organic dairy farms in the Northeast region. The cows on 3 farms continued feeding their regular supplements which was formulated to yield 14.8% CP (low group), whereas the cows on other 3

farms fed a diet with 19.3% CP (high group) where the CP was altered in their diet by using more organic barley and roasted soybean mix. High group produced 21% more milk (24.1 vs. 19.9 kg/d) than low group. Milk fat% and fat yield increased in the low group whereas high group had greater milk protein% and yield. The authors recommended that increasing the level of CP in the diet through supplementation may be an effective strategy to improve milk production throughout the summer grazing season.

Bargo et al. (2003) reviewed different types of supplements on pasture and concluded that milk yield increased linearly with the increase of concentrate, ranging from 1.2 to 10 kg DM/d. The overall milk response was reported to be 1 kg milk/kg of starch or fiber-based concentrate (i.e., corn, barley, beet pulp, citrus pulp) compared to the diets containing no supplements which is also consistent with Reis et al. (2000) who evaluated 3 diets (0, 5, and 10 kg of concentrate/d) and found that pasture supplementation with 10 kg/d of concentrate had higher milk yield, milk protein concentration, and total VFA concentration.

Effect of Flaxseed Supplementation on Production Performance of Dairy Cows

Few studies have been conducted previously using LinPRO-R as an extruded flaxseed-based supplement on productive and reproductive performance, milk fatty acid profile, and enteric CH_4 emissions in confined dairy cows (Judy et al., 2019; Moats et al., 2018; Swanepoel and Robinson 2019a; Swanepoel and Robinson 2019b). Several studies compared other different forms of flaxseed in the diet of dairy cattle (Mustafa et al., 2003a; Mustafa et al., 2003b; Gonthier et al., 2004a; Gonthier et al., 2004b; Gonthier et al., 2005; Da silva et al., 2007; Petit and Cortes 2010; Cortes et al., 2010; Cortes et al., 2011; Neveu et al., 2014; Hawkins et al., 2013; Isenberg et al., 2019)

Studies have demonstrated inconsistent results on intake and apparent total tract digestibility of nutrients, and milk yield and components when fed different inclusion of either ground flaxseed or extruded flaxseed to the dairy cows. For instance, Isenberg et al. (2019) observed no difference for DMI, milk yield and milk composition when cows fed 10% ground flaxseed (of the diet DM) to Jersey cows compared to control during grazing season. Intake of neutral detergent fiber (**NDF**) and acid detergent fiber (**ADF**) were greater whereas total tract digestibility of NDF was lower in cows fed ground flaxseed compared to control. Contrast to these findings, Resende et al. (2015) reported a linear decrease in DMI, milk yield, and digestibility of nutrients when incremental amounts (0, 5, 10, and 15% of the diet DM) of ground flaxseed was fed to the lactating Jersey cows. When fed the same incremental amounts of extruded flaxseed, DMI and milk yield did not differ across the diets, but concentration of milk fat was reduced linearly or quadratically. However, no differences were observed for yield of milk components (Ferlay et al., 2013).

Lerch et al. (2012b) conducted 2 experiments comparing an extruded flaxseed diet and different forms of rapeseed (to provide 2.5-3.0% oil of diet DM) in a grass-based diet. In experiment 1, milk yield did not differ between treatments, but in experiment 2, milk yield was reduced in extruded flaxseed diet compared to rapeseed diet. Also, feeding extruded flaxseed decreased milk fat% compared to control. Oeffner et al. (2013) used 4 different inclusion rates of extruded flaxseed (0, 0.91, 1.81, 2.72 kg/d), and 1.81 kg/d of ground flaxseed. The study lasted 10 weeks and treatments were top dressed. No effects were found for milk yield and milk components among diets.

Studies conducted using LinPRO-R also showed discrepant results. Swanepoel and Robinson (2019a) evaluated LinPRO-R in a commercial farm using 315 early lactation

multiparous dairy cows. Three different treatments of LinPRO-R were tested (DM basis): 0 % (NoLin), 2.5% (LoLin), and 5.0% (HiLin). whereas the intake of DM and the concentration of milk fat and true protein did not differ across the diets, milk yield and yield of milk components increased linearly. Overall, the authors concluded that the productive performances of early lactation dairy cows can be improved with feeding 2.5% LinPRO-R in the diet. When fed same inclusion LinPRO-R to the late lactation cows (Swanepoel and Robinson, 2019b), concentration of milk fat increased linearly, whereas lactose quadratically tended to be greater with no effect on the yield of milk and milk components (i.e., fat, true protein, lactose). The effects of LinPRO-R on production performance in early or late lactation cows appeared to be different in the last 2 studies suggesting that feeding LinPRO-R may be beneficial for early lactating cows. Consistent with the results of Swanepoel and Robinson (2019b), Judy et al. (2019) also reported no changes in milk yield and milk components when cows were fed 10.2% diet DM LinPRO-R compared to a control diet.

Moats et al. (2018) investigated 4 diets using different forms of flaxseed: 1) control diet without flaxseed 2) 11.4% diet DM non-extruded flaxseed 3) 11.4% diet DM LinPRO-R 4) 11.4% extruded flaxseed and tannin containing fava beans. Dry matter intake (DMI) was lower in all diets containing flaxseed. Milk yield was higher in LinPRO-R compared to non-extruded flaxseed, whereas no effects were observed for milk components. Overall, milk yield tended to be greater in cows fed flaxseed diet relative to control diet.

Comparisons were made among different forms of flaxseed (whole flaxseed, ground flaxseed, extruded flaxseed, micronized flaxseed, heated flaxseed, and unheated flaxseed) in many studies. Gonthier et al. (2004a, 2005) evaluated 3 forms of flaxseed: raw flaxseed, micronized flaxseed, extruded flaxseed, and a control diet. The intake of DM, organic matter

(**OM**), NDF, and ADF did not differ in all 3 forms of flaxseed compared to control. Except ADF, which was reduced in all flaxseed diets, ruminal digestibility of DM, OM, and NDF were also similar when compared to all flaxseed diet vs. control. However, the digestibility of DM, OM, and ADF were greater in cows fed extruded flaxseed than micronized flaxseed diet. The authors reported their inability to detect a statistical difference in milk yield and components between control and flaxseed diets due to the small number of animals in the studies. Milk yield and energy corrected milk (**ECM**) were reported to be lower in cows fed flaxseed. Specifically, flaxseed cows produced 1.8 kg less milk than those fed the control diet, and extruded flaxseed cows yielded 1.6 kg less milk than cows offered micronized flaxseed. Also, the extruded flaxseed diet yielded less milk fat compared to the other diets which was due to the reduced concentration of milk fat. Moreover, the concentration of milk protein was lower with feeding extruded flaxseed, while lactose concentration remained unchanged across diets. The authors concluded that adding up to 12.6% (diet DM) of different types of flaxseeds did not have any negative effect on intake and digestibility of nutrients, but feeding extruded flaxseed compared to other forms of flaxseed affect production performance in late lactation dairy cows.

Petit and Cortes (2010) fed an isoenergetic diet with either 1) 2.12% (diet DM) calcium salts of palm oil 2)7.2% (diet DM) whole flaxseed 3) 7.2% (diet DM) ground flaxseed or 4) 3.6% (diet DM) whole flaxseed and 3.2% (diet DM) ground flaxseed. Intake of DM was higher when the whole flaxseed or combination of whole flaxseed and ground flaxseed were fed compared to the diet fed only ground flaxseed. Except lactose which was decreased in control diet compared to 3 other diets, milk yield or composition did not differ across the treatment.

Beauchemin et al. (2009) compared 4 different fat sources: Ca sources of long chain FA, crushed sunflower seeds, crushed flaxseed, and crushed canola seed. Oilseeds supplied 3.1 to

4.2% fat to the diets. No differences were found in milk yield and milk components across diets. Prado et al. (2016) reported an increase in milk yield and yields of milk components in dairy cows fed 1.1% (diet DM) of Ca salts of palm oil compared with a control diet or a diet containing 2.2% (diet DM) of whole flaxseed. A meta-analysis was performed by Leduc et al. (2017) to determine the efficacy of different forms of flaxseed on production performance in dairy cows. The results indicated that whole and ground flaxseed yielded the highest milk and ECM, and a greater feed efficiency compared to other forms of flaxseed (i.e., flax oil, intact or extruded whole flax, protected flax, or flax hulls).

Neveu et al. (2014) fed corn and barley with or without 10% diet DM of extruded flaxseed and reported a greater intake of DM, OM, CP, and NDF in cows fed extruded flaxseed compared to no flaxseed diets. Similarly, no difference was observed for total tract digestibility of DM, OM, and NDF between the diets but a tendency was observed for CP digestibility to be greater in cows fed extruded flaxseed diets than those fed no flaxseeds. The yield of milk fat was greater in extruded flaxseed diets, and a tendency for ECM and 4% FCM were observed to be higher when cows were fed extruded flaxseed with either grain sources compared to no flaxseed diet.

Mustafa et al. (2003a) observed no effects on milk components when ground unheated or micronized flaxseed were fed to early lactation dairy cows at a rate of 7% in the DM. However, concentration of milk fat was reduced in cows fed flaxseed diet compared to control. Isenberg et al. (2019) fed 10% (diet DM basis) ground flaxseed to grazing dairy cows and found no difference in DMI and milk yield and components relative to the control diet. However, the apparent total-tract digestibility of OM was lower and that of NDF was higher in cows fed ground flaxseed.

Petit et al. (2005) investigated 2 levels (16 vs 18%) of CP in the diet with or without whole flaxseed. Milk yield did not differ between flaxseed and no flaxseed diet but tended to be higher when cows fed flaxseed with high protein diet. However, total DMI, concentration of milk protein and lactose, as well as the digestibility of nutrients (I.e., DM, CP, ADF, NDF) decreased in cows fed flaxseed than those offered no flaxseed. The authors concluded that decreased DMI in flaxseed fed cows may have affected milk components.

Da silva et al. (2007) compared diets containing whole flaxseed and ground flaxseed with or without monensin. No effects of monensin or interaction (flaxseed × monensin) was observed for DMI or milk yield. However, milk yield tended to be greater in cows fed ground flaxseed than those fed whole. Milk composition did not differ across the treatments. Whereas the digestibility of DM, and NDF did not change between whole and ground flaxseed diet. In contrast, digestibility of ADF was greater and CP and ether extract digestibility were lower in whole flaxseed compared to ground. Romero et al. (2017) evaluated diets containing pelleted and non-pelleted ground flaxseed with or without monensin. No treatments effects were observed on milk yield and milk components, as well as intake and digestibility of nutrients.

Flaxseed oil was also used in some studies to evaluate milk production performance and milk FA profile. Moallem et al. (2012) investigated different amounts (110 ml/d and 220 ml/d) of flaxseed oil infusion and a control diet with no flaxseed oil. Except lactose, which tended to be greater in control diet, no effects were observed for DMI, yield of milk and components in flaxseed oil diet compared to control. Cortes et al. (2010) fed 4 diets; 1) no flaxseed 2) 4.2% whole flaxseed 3)1.9% calcium salts of flaxseed oil 4) whole flaxseed and 0.8% calcium salts of flaxseed oil. Except milk fat concentration that was decreased when cows were fed flaxseed oil

compared to control, no other changes were observed for milk yield and components across the diets.

Overall, it appears that the production performance of cows is inconsistent with different flaxseed supplementation in different feeding and management schemes. The response largely depends on a variety of other factors for instance, flaxseed inclusion level, stage of lactation, type of forages and concentrates in the basal diet, and most importantly the way flaxseeds are processed. Therefore, this study could give some insights on whether this extruded flaxseed-based supplement LinPRO-R can improve production performance in dairy cows during the grazing season.

Effect of Flaxseed on Ruminal fermentation Profile

It is well established that flaxseed supplementation increases PUFA in the diet which may have an adverse effect on rumen microbes particularly on cellulolytic microorganisms (Maia et al., 2007); therefore, fiber digestion is often interrupted (Jenkins, 1984, 1993). There are 2 ways in which fat supplements affect the ruminal microorganisms: 1) FA disrupts the cellular membrane of microorganisms 2) fat sources reduce the availability of cations such as Ca and Mg (Jenkins, 1993). Production of VFA and the acetate-to-propionate ratio may also decrease due to these negative effects (Jenkins, 1993).

Neveu et al., (2013) fed 2 different forages: concentrate ratio (60:40 and 40:60) with or without 9% (diet DM basis) extruded flaxseed. Total VFA concentration did not change across the diet. Similarly, concentration of acetate, propionate and butyrate and the acetate: propionate did not differ between cows fed flaxseed diet than those fed no flaxseed diet.

In another experiment, Neveu et al. (2014) fed corn and barley with or without 10% (diet DM basis) extruded flaxseed. Flaxseed diet did not affect NH_3-N or total VFA concentration compared to control. Propionate concentration decreased with feeding flaxseed diet, while butyrate increased. However, concentration of acetate or the acetate-to-propionate ratio remained unchanged.

Total VFA concentration did not differ when cows fed control, raw, micronized, and extruded flaxseed at 12.6% diet DM. Whereas molar proportion of acetate decreased in flaxseed diet compared to control, propionate increased. Also, the acetate-to-propionate ratio decreased in cows fed flaxseed than control (Gonthier et al., 2004a)

Beauchemin et al. (2009) fed 4 diets: 1) control diet with calcium salts of long chain FA 2) crushed sunflower seed 3) crushed flaxseed 4) crushed canola seed. The diets were formulated to provide 3.1 to 4.2% fat (diet DM basis). The concentration of total VFA, acetate, propionate and the acetate-to-propionate ratio did not differ across the treatments while butyrate decreased in crushed flaxseed compared to control.

Isenberg et al. (2019) investigated a control and a ground flaxseed diet (10% of the diet DM) fed to Jersey cows during grazing season. Except butyrate which tended to decrease in flaxseed diet, total VFA concentration and the concentration of acetate, propionate, and butyrate did not differ between diets. However, the acetate-to-propionate ratio decreased in cows fed flaxseed.

Moats et al. (2018) evaluated different forms of flaxseed supplements (extruded, non-extruded, flaxseed with peas, tannin containing fava bean with extruded flaxseed). Concentration of acetate increased in extruded flaxseed diet compared to the diet fed extruded flaxseed

combined with tannin containing fava bean. The concentration of other individual VFA did not change across the diets.

A study done by Resende et al. (2015) in which cows were fed incremental amounts (0, 5, 10, and 15% of the diet DM) of ground flaxseed and reported linear decreases in the ruminal molar proportions of acetate and butyrate, whereas the molar proportion of propionate increased linearly, thus decreasing the acetate: propionate ratio.

Soder et al. (2012) used a continuous culture fermenter to evaluate an orchardgrass based diet replaced with 0, 5, 10, and 15% of the diet DM with ground flaxseed. The concentrations of acetate, propionate, increased linearly in flaxseed diets whereas butyrate decreased. However, total VFA concentration and the acetate: propionate ratio was not affected by diets (Soder et al., 2012).

Flaxseed supplementation yields a variety of responses to ruminal volatile fatty acid concentration which are subjected to change depending on the ingredients of the diet. However, based on the studies mentioned above, it appears that the concentration of butyrate decreased and total VFA concentration remained unchanged with the inclusion of ground flaxseed in the diet of dairy cows. However, feeding extruded flaxseed at different levels may not change the ruminal fermentation profile.

Milk Fatty Acids Composition in Cows on Pasture

Nutrition is considered as one of the most striking factors and an important practical tool for producers to change the yield and composition of milk fat (Bauman and Lock, 2006). In the past years, modifying the FA composition of milk through dietary manipulation has been a great interest of many scientists. In general, pasture itself and its botanical composition drive the

changes in milk FA. For example, red clover contains the enzyme polyphenol oxidase that protects glycerol-based lipid from lipolysis which results in more PUFA in milk (Lee et al., 2009). Dewhurst et al. (2003) compared legume silage versus grass silage on milk FA composition. Cows fed legume silage had greater concentration of PUFA, particularly ALA and lower proportion of 16:0. In general, milk from grazing ruminants have lower proportion of saturated FA and higher proportion of *trans* FA, polyunsaturated FA, and *cis*-9 *trans*-11 CLA compared to the feeding of conserved forage (Chilliard et al., 2007). Additionally, providing diverse pasture instead lowland reduces SFA (especially 4:0 to 16:0) concentration and particularly increased CLA and ALA in milk (Leiber et al., 2005; Mohammed et al., 2009).

Decaen and Ghadaki (1970) reported 4 times higher CLA in milk when cows grazed fresh grass compared to feeding hay or concentrate indoor. Chilliard et al. also (2000) reviewed different feeding strategies and their influence on milk CLA concentration and found that CLA in milk increased up to 0.8-1.6% when cows grazed young grass or fresh pasture.

Agenas et al. (2002) conducted a 4 wk study using 44 multiparous lactating cows to investigate the effect of pasture turn out with dietary fat supplementation (soy oil). Long chain fatty acid increased, and the concentration of *de*-novo synthesis of fat (4:0-14:0) decreased in milk during pasture turn out and fat supplementation.

Coppa et al. (2011) compared 3 feeding systems: 1) hay and concentrate-based diet indoor, 2) weekly rotation grazing on a diverse pasture with 74 species, and 3) continuous grazing with 31 species. Milk from cows raised in the indoor system produced the lowest concentration of monounsaturated FA (**MUFA**) and PUFA with the *cis* and *trans* isomer of 18:1, *trans*-11-C18:1, and CLA isomer, whereas the concentration of PUFA was constant throughout the season in the rotational grazing system.

Pasture usually provides 1-3% FA on DM basis with the highest value observed in autumn and spring (Bauchart et al., 1984; Elgersma et al., 2006). It is commonly found that CLA was 2-3-fold higher in cows grazing fresh pasture but as soon as the plants matured, the concentration of CLA starts to decline (Kelly et al., 1998; Dhiman et al., 1999). The observed effect cannot be exclusively attributed to the FA composition and the supply of PUFA from grass. Therefore, it is possible that additional factors are involved in enabling the synthesis of *trans-11* 18:1 within the rumen. The CLA in milk is the aftermath of the rumen biohydrogenation of *trans*- 18:1 FA isomer by delta-9 desaturase. There are many factors that influence delta-9 desaturase enzymatic activity such as diet composition, intake of 18:0 FA, and most importantly rumen microbial population.

Several studies compared the effect of low versus high pasture allowance on milk FA profile and reported no major changes in milk FA (Stanton et al., 1997; Wales et al., 1999; Stockdale et al., 2003). The forage conservation process especially during hay making and wilting before ensiling results in significant losses of ALA (Dewhurst et al., 2006). Few studies have shown beneficial effects to protect n-3 FA especially ALA and when formic acid is added during enisling of grass silage (Dewhurst and King, 1998, Shingfield et al. 2005).

Results from a field survey indicated that *cis*-9, *trans*-11 18:2 CLA and ALA reduced following this order: alpine pasture> first use permanent grassland pasture> second use of permanent grassland pasture > pasture for temporary use> grass silage> hay > corn silage (Lucas et al., 2006). There are also seasonal changes in milk FA (Frelich et al., 2012), and it is not obvious that pasture always changes milk FA. For example, Lawless et al. (1998) and Kelly et al. (1998) reported that ALA remained less than 1% even when the cows were transitioned from indoor feeding to pasture.

Atkins et al. (2020) investigated the effect of grazing period on milk FA composition. Fifty-six Holstein cows were assigned to 4 different treatments as follows: 1) Control: Cows were offered TMR ad libitum indoor, 2) Early grazing: Cows were offered 6 h grazing time after morning milking then fed partial total mixed ration (pTMR) indoor, 3) Delayed grazing: Cows returned to the barn for 1 h after morning milking followed by 6 h of grazing, and 4) Restricted pTMR: Cows were offered 6 h grazing time after the morning milking and then fed 75% of pTMR indoor. The restricted treatment led to the lowest concentrations of C10:0, C14:0, and C15:0, and greater MUFA in milk compared to early grazing. The concentration of ALA was greater in all the 3 treatments with pasture compared to control. Authors concluded that 6 h of pasture allocation can enrich milk with ALA but the concentration was below 0.5% of the total amount of milk fat.

In addition to the pasture and nature of forages, the changes of FA in milk are observed when cows are supplemented with different types of concentrates such as oilseeds, as well as protected or unprotected fish or vegetable oil (Chilliard et al., 2000). Dhiman et al. (1999) conducted a series of 4 experiments to evaluate different diets on milk CLA concentration. In experiment 1, the inclusion of high oil corn and high oil corn silage in the diet to provide an extra 1% of fat did not affect CLA concentration in milk. In experiment 2, The CLA concentration of milk increased linearly with the proportion of grazed herbage included in the diet of dairy cows. In experiment 3, The concentration of CLA was 500% higher in cows that were allowed to graze on permanent natural pastures, as compared to cows that were fed TMR consisting of conserved forage and grain in a 50:50 ratio.

In conclusion, dietary manipulation is one of the best strategies, easy to implement, and can achieve our desired changes of FA in milk within the shortest possible time. However, more

research is warranted to see which combination in terms of pasture and dietary concentrate formulations are cost-effective and suitable for the dairy producers.

Flaxseed supplements: A strategy for enriching milk with n-3 fatty acids

The n-3 FA are important for domesticated animals to maintain their normal physiological process (Palmquist, 2009). Consumers are becoming increasingly aware of the potential health benefits of PUFA such as n-3 FA, which humans are unable to produce. In fact, the nutritional composition of milk fat has been discussed for a long time due to its lower concentration of fatty acids that are considered beneficial for human health in comparison to fats derived from vegetables or animals. Therefore, there is an increasing effort to alter dairy cow diets to enhance PUFA content of milk. However, the problem is further exacerbated by the fact that ruminal bacteria alter the dietary FA composition by isomerization and biohydrogenation of unsaturated FA (Harvatine and Allen, 2005).

In dairy nutrition research, supplements containing oilseeds and protected fats are the most common strategies to change the FA profile in milk that are beneficial to human health. Flaxseed is a well-known source of n-3 FA with a composition of 50% ALA (Moallem et al., 2012). Research conducted on the use of ground flaxseed, extruded flaxseed, or flaxseed oil increased the concentration of n-3 FA especially ALA and declined in the ratio of n-6 to n-3 in milk fat (Ferlay et al., 2013; Isenebrge et al., 2019; Resende et al., 2015; Brossillon et al., 2018).

da Silva et al. (2007) fed a diet of ground flaxseed and whole flaxseed with or without monensin. Ground flaxseed had greater concentrations of CLA and ALA in milk but 16:0 and 17:0 decreased. Similarly, the SFA and medium chain FA (**MCFA**) reduced and PUFA increased when the cows were fed ground flaxseed compared to whole flaxseed. The

concentration of CLA increased and SFA reduced with cows were fed monensin with either of the flaxseed. The modification of milk fatty acid composition through flaxseed processing and monensin supplementation has been shown to potentially enhance its nutritional value for consumers.

Moallem et al. (2012) evaluated the transfer efficiency of ALA into milk by abomasal infusion of flaxseed oil. Three diets were used as follows: 1) Control:110 mL of water/d, 2) 110 mL of flaxseed oil/d, and 3) 220 mL of flaxseed oil/d. The concentration of ALA in milk increased in both low and high flaxseed oil by 1.68 and 3.09 %, respectively compared to control. The concentration of CLA was greater in high flaxseed oil diet compared to control but did not differ with low flaxseed oil. Low flaxseed oil and high flaxseed oil reduced 16:0 FA in milk by 3.6% and 5.25% respectively, compared to control.

Neveu et al. (2013) supplemented extruded flaxseed to a high or low forage (corn silage diet (diet supplemented with corn silage and alfalfa haylage as forage sources) with in a 2 × 2 factorial study. The SFA decreased, whereas MUFA increased when extruded flaxseed or low forage diets were fed. Feeding extruded flaxseed or low-forage diets increased PUFA compared to other diets. The ALA content increased by 100%, and CLA content increased by 54% when cows were supplemented with extruded flaxseed. In a subsequent study, Neveu et al. (2014) investigated grain sources (corn vs. barley) with extruded flaxseed. Supplementation with extruded flaxseed increased the content of ALA and CLA by 60 and 29%, respectively, and reduced the concentration of SFA. The authors stated that the changes in milk FA were mainly due to the inclusion of extruded flaxseed.

Petit and Cortes (2010) fed whole or ground flaxseed and a control diet in the first half of lactation. Both flaxseed diets enhanced ALA concentration in milk, whereas the control diet had

greater concentrations of 16:0 and lower concentrations of 18:0 and *cis*-918:1 compared to flaxseed.

A meta-analysis by Leduc et al. (2017) evaluated the n-3 FA transfer efficiency of 6 different forms of flaxseed. Protected flax and flax hulls in the diet led to greater concentrations of ALA in milk. Additionally, mechanically processed whole flaxseed (rolled or ground), protected flax, and flax hulls had the greatest transfer efficiency of n-3 from the diet to the milk.

Cortes et al. (2011) fed 6 diets: 1) control without flax hull or flax oil 2) 15.9% flax hull on DM basis 3) control with 250 g/d of abomasal infusion of flax oil 4) control with 500 g/d of abomasal infusion of flax oil 5) 15.9% flax hull with 250 g/d of abomasal infusion of flax oil 6) 15.9% flax hull with 500 g/d of abomasal infusion of flax oil. It was reported a greater proportion of 18:0 and *cis*-918:1 in milk when cows were fed 15.9% (DM basis) flax hulls with flax oil compared to control with no flax hull. Flaxseed oil led to a lower n-6 to n-3 ratio compared to flax hulls which suggest a greater modification of FA in rumen for flax hulls than flax oil. The ALA concentration was greater both in flax hull and flax oil diets, but no interaction (flax hull × flax oil) was reported.

Romero et al. (2017) fed pelleted and non-pelleted ground flaxseed with or without monensin. The addition of monensin in the diet increased CLA concentration by 47% whereas feeding pelleted flaxseed decreased the SFA especially 14:0, 18:0, 20:0, and 24:0 and enhanced the CLA and PUFA concentration by 70 and 25%, respectively. Zhang et al. (2005) observed a linear increase in LCFA, MUFA, and PUFA when the lactating ewes were fed 18 and 26% of flaxseed meal (diet DM basis). In contrast, a linear decrease was observed in SFA concentration. Additionally, the concentration of CLA and ALA increased by 73 and 43%, respectively.

Mustafa et al. (2003a) fed a control diet without flaxseed, unheated flaxseed, and micronized flaxseed at 7% of the diet DM to early lactating dairy cows. The unheated and micronized flaxseed lowered the concentrations of saturated FA and monounsaturated FA and increased the concentration of long-chain FA (18:0 to 18:3). However, the changes of the extent of PUFA concentration in milk were minor while feeding micronized vs unheated flaxseed.

Our previous studies on top-dressed ground flaxseed with pTMR at a rate of 0% or 10% (DM basis) changed most SFA and UFA. Supplementing 10% ground flaxseed increased the concentration of ALA, Σn-3 FA, and ΣC18 FA (Isenberg et al., 2019). Resende et al. (2015) fed incremental amount (0, 5, 10, and 15% diet dry matter) of ground flaxseed to dairy cows receiving TMR with a forage to concentrate ratio of 63:37. Cows fed ground flaxseed had a linear and quadratic increase in milk *trans*-11 18:1, ALA, CLA, and total n-3 FA. Additionally, the milk FA ratio of n-6 to n-3 decreased linearly with feeding ground flaxseed.

Swanepoel and Robinson (2019b) compared 3 diets with different amounts of LinPRO-R (i.e., NoLin: 0% DM, LoLin: 2.5% DM, and HiLin: 5% DM) to the mid-to-late lactation dairy cows in a commercial farm setting. Milk ALA concentration increased linearly whereas highest milk CLA was observed when fed LoLin compared to HiLIN.

Effect of Flaxseed Supplements on Enteric Methane Emissions

Enteric CH_4 is produced as a by-product of fiber digestion and feed fermentation in the rumen of cattle and considered as a primary source of on-farm greenhouse gas emissions (Veysset et al., 2010). Enteric fermentation contributes 27% of the total CH_4 emissions (EPA, 2021). Regardless of any ideal feeding system, production of CH_4 varies from animal to animal, and represents 2 to 12% of gross energy intake (Johnson and Johnson, 1995; Martin et al., 2008);

therefore, decreasing CH_4 emissions may increase feed efficiency and productivity of ruminants. It is well known that the ingredient composition of the diet and DMI are main drivers of the amount of CH_4 produced in cows (Johnson and Johnson, 1995). Additionally, CH_4 output usually increases as the digestible carbohydrate in the diet increases. Therefore, dietary manipulation is considered as one of the effective strategies to reduce enteric CH_4 emissions (Monteny et al., 2006). Dietary lipids, particularly vegetable oils, can lower CH_4 emissions. However, the anti-methanogenic effect of supplemental fat depends on several factors such as the type and the amount of FA in the diet, full fat oilseed versus oil, ground, or crushed vs extruded, and type of forage and grain sources (Beauchemin et al., 2008; Hristov et al., 2013; Knap et al., 2014).

It has been shown that extruded flaxseed, flaxseed oil, or crushed flaxseed when added to the diet as a fat supplement decreased CH_4 emission from dairy cows (Martin et al., 2008; Beauchemin., 2009; Martin et al., 2016; Boland et al., 2020;). There are a few different mechanisms that have been postulated to explain how flaxseed serves as an anti-methanogenic feed supplement. Firstly, including flaxseed in the diet can replace carbohydrates, which slows ruminal fermentation and consequently reduces fiber digestion (Huhtanen et al., 2009). Second, it is possible that oil from flaxseed has a direct impact on ruminal methanogens and thirdly, the act of supplementing may enhance the degree of biohydrogenation, thus serving as a hydrogen sink (Martin et al., 2010; Knap et al., 2014).

Martin et al. (2008) fed 3 diets: 1) a control diet without flaxseed 2) 12.4% (DM basis) whole flaxseed and 3) 14.8% (DM basis) extruded flaxseed. Each diet was formulated using different inclusion of flaxseed to provide 5.7% (diet DM basis) total FA in each diet. Daily CH_4 production, and CH_4 intensity were reduced by 38%, and 23%, respectively when cows were fed extruded flaxseed diet compared to CTRL. Total DMI decreased, and 5% reduction of DM, and

OM digestibility, and 25% reduction in NDF digestibility were observed in extruded flaxseed diet which also resulted in a negative impact on milk yield and components.

In another study, Martin et al. (2016) investigated extruded flaxseed supplementation at incremental levels (0, 5, 10, 15% of the diet DM) with hay or corn silage. The diet fed with flaxseed decreased CH_4 output linearly which was corroborated by Almeida et al. (2023), where it was demonstrated that CH_4 production tended to decrease linearly with feeding incremental amount of ground flaxseed to Jersey cows. Soder et al. (2012) investigated incremental amounts (0, 5, 10, and 15% diet DM) of ground flaxseed replacing orchardgrass in continuous culture. CH_4 production decreased linearly as the flaxseed inclusion increased from 0 to 15%. Contrarily, our previous grazing study with feeding 0 versus 10% ground flaxseed (DM basis) had no effect on enteric CH_4 emissions (Isenberg et al., 2019).

Judy et al. (2019) compared 2 diets (control vs LinPRO-R) with different concentrations of ALA (0.14 vs. 1.2% of the diet DM). The LinPRO-R diet contained 10.2% (diet DM) of LinPRO-R. No effect was observed on enteric CH_4 emissions between the diets, suggesting that increasing ALA concentrations may not have any effect on ruminal methanogenesis. Beauchemin et al. (2009) fed crushed flaxseed (9.32% DM) and compared with control, crushed sunflower (10.55% DM), or canola seeds (9.32% DM). It was observed that the CH_4 output was reduced by 18% when crushed flaxseed was fed. Overall, based on the literature findings, there are discrepancies of CH_4 production between different forms of flaxseed, and it has been consistently reported that ground, crushed flaxseed, or flaxseed oil reduces CH_4 output, but the response is highly dose dependent.

Effect of Flaxseed Supplementation on Energy Utilization

Research on energy metabolism carried out over the last 50 years has uncovered many important aspects of energy utilization in lactating dairy cows. Energy balance studies are limited in dairy cows particularly with feeding flaxseed, but others have tested different lipids or FA supplementation on energy utilization in dairy cows (Andrew et al., 1990; Weiss and Wyatt, 2004; Harvatine and Allen, 2006; Morris et al., 2020; Razzaghi et al., 2022). Fats are commonly used to increase the energy density of the diet which is being utilized as energy supplies after the absorption in small intestine with an average enthalpy of 9.3 Mcal/kg of DM (NRC, 2001), implying a direct transfer of energy to the milk fat (Rico et al., 2014; Boerman et al., 2015). The incorporation of dietary FA into milk is also known to increase the efficiency of converting metabolizable energy (ME) into milk energy, as lipid-derived energy supplies play a crucial role in this process. (Hammon et al., 2008). Indeed, the dietary manipulation of forage to concentrate ratio, or supplementing unsaturated FA through dietary lipids affects N and energy utilization in ruminants (Morris et al., 2020).

Judy et al. (2019) fed a control diet and 10.2% LinPRO-R (diet DM) to supply increasing concentration (1.20% diet DM basis) of ALA in the diet. Digestible energy of control diet and LinPRO-R diet was 2.73 and 2.80 Mcal/kg, respectively which was calculated based on the nutrient digestibility of each diet and using a heat combustion values 4.2 Mcal/kg for starch and NDF, 5.6 Mcal/kg for CP, and 9.4 Mcal/kg for fat (NRC, 2001). Total DMI, and digestibility did not differ between diets, thereby no effects on digestible energy intake. Similarly, the diets did not have any effect on CH_4 energy and heat production. It appears that inclusion of LinPRO-R in the diet did not improve energy utilization of the dairy cows.

Estimation of Herbage Intake

Accurate estimation of herbage intake is very difficult in practical and experimental settings. To date, different methods have been used to estimate herbage intake in grazing ruminants such as N-alkanes and ^{13}C techniques (Garcia et al., 2000; Malossini et al., 1995), chromium oxide (**Cr_2O_3**) as an external marker (Smith and Reid 1954; Malossini et al., 1995; Bargo et al., 2002a), titanium oxide in combination with in-vitro organic matter digestibility, acid detergent lignin (**ADL**), and indigestible neutral detergent fiber (**iNDF**; Hellwing et al., 2015). Herbage intake can also be estimated using energy requirements of animals for milk production and maintenance (Smit et al., 2005), and fecal output evaluation using a pulse-dose marker, and herbage disappearance rate (Macoon et al., 2003). Subtracting post-grazing biomass from pre-grazing is an indirect way to estimate herbage intake but this approach provides estimations for a group of cows rather than individual intake, which is a limitation (Bergo et al., 2002a, b).

Hellwing et al. (2015) compared a total of 9 methods to calculate herbage intake of grazing dairy cows. There were 3 different methods (i.e., based on animal performance, ^{13}C techniques using in-vitro organic matter digestibility, discrimination factor, and titanium oxide) that yielded similar output when the cows were fed indoors during the day and grazed all night. However, the authors reported a very low correlation among different methods which was consistent with what others found when compared pulse dose marker vs. herbage disappearance method, or n-alkanes vs. Cr_2O_3, (Macoon et al., 2003; Malossini et al., 1996)

A comparison between n-alkanes and ^{13}C technique was made by Garcia et al. (2000) and found that combining these 2 methods together can result in better estimation of herbage intake which is consistent with the findings of Wright et al. (2019) who reported that n-alkanes

technique was accurate regardless of herbage management and seasonal changes. Moreover, Smit et al. (2005) investigated 3 different techniques: 1) pre-grazing and post-grazing mass and a regrowth between the timepoints; 2) naturally and synthetic n-alkanes, and 3) animal energy requirement. Pre-and post-grazing technique had the largest variation in different experimental years whereas n-alkanes method yielded less variable result. The authors suggested that n-alkanes method can be used for direct estimation of herbage intake which was corroborated with Malossini et al. (1996) who also reported N-alkanes as labor efficient and easy method compared to others.

Chromium oxide in combination with in-vitro dry matter digestibility (IVDMD) has also been used as an external marker to calculate the fecal DM output and estimate herbage intake (Smith and Reid 1954; Bargo et al., 2002a). Smith and Reid (1954) conducted a series of 5 experiments using different doses and forms (gelatin capsule or in concentrate feed) of Cr_2O_3 over 3 grazing seasons. In experiment 1, feces were collected at 2 h intervals on day 2 and day 6^{th} and 6 h interval for the rest of 5 days. In the next 3 experiments, feces were collected at 6 a.m. and 4 p.m. The results from the first experiment demonstrated that there was some intraday variation of chromium (**Cr**) concentration in the feces at different times of the day and night. However, the authors were able to get a satisfactory Cr excretion and fecal output result when feces samples were taken 6 a.m. and 4 p.m. Therefore, it has been recommended that researchers may consider fecal sampling at some specific time (morning and afternoon) of the day throughout the experiment to minimize the variation across samples. Interestingly, the authors were able to retrieve reliable pasture intake data even from sampling of feces from a single day. However, one of the drawbacks of the Cr_2O_3 approach is that it assumes all cows have the same digestibility.

Macoon et al. (2003) estimated herbage intake based on 3 methods:1) animal performance by considering net energy requirements for lactation 2) fecal output using chromium mordanted fiber as an external marker and 3) disappearance of herbage. The pulse dose marker resulted in higher herbage intake than the other methods. However, the difference of herbage intake estimation between animal performance and fecal output was lower compared with the pulse dose marker approach. Therefore, it was reported that herbage disappearance method was easy and economical and may be used for estimating herbage intake in grazing dairy cows.

Even if there are a several different ways to estimate herbage intake, each approach comes with its own set of benefits and challenges. Depending on the type of measurements that are needed for each scenario, it may be necessary to consider and make use of a variety of different approaches.

Conclusion

Inclusion of flaxseed in the dairy diet provides an array of benefits. This is one of the effective strategies for consumers seeking nutrient-dense heart-healthy FA, especially milk n-3 FA. Besides improving milk quality, feeding flaxseed is beneficial for reproductive health and overall well-being of the cows. If feeding flaxseed is cost-effective, industry adoption of developing n-3 specialized milk or milk products could pave the way for more profitable dairy farms.

CHAPTER II. EFFECTS OF FLAXSEED-BASED FEED SUPPLEMENT ON PRODUCTION PERFORMANCE, ENERGY UTILIZATION, MILK FATTY ACID PROFILE, AND ENTERIC METHANE EMISSIONS IN JERSEY COWS GRAZING MIXED GRASS-LEGUME PASTURE

INTRODUCTION

The nutritional composition of milk fat has been discussed for a long time due to its lower concentration of fatty acids (**FA**) that are considered beneficial for human health (Kennelly, 1996). Consumers are becoming increasingly aware of the potential health benefits of omega-3 (**n-3**) FA especially *cis*-9, *cis*-12, *cis*-15 18:3 (i.e., α-linolenic acid = **ALA**) and conjugated linoleic acid (**CLA**) such as *cis*-9, *trans*-11 18:2 which have been shown to reduce the incidence of inflammation, hypertension, arthritis, coronary heart disease, and cancer (Williams 2000; Simopoulos, 2002; McCrorie et al., 2011; Dilzer and Park, 2012). The n-3 FA have also been recognized to improve normal growth, immunity, vision, and brain development (Williams, 2000; Connor, 2000). Therefore, increasing efforts have been made in the last decades to find suitable and cost-effective strategies to change the FA composition in milk of cows that are beneficial to human health. Dietary supplementation with flaxseed has been suggested as an excellent method to enrich milk with beneficial polyunsaturated FA (**PUFA**) especially ALA and CLA. Flaxseed is a well-known source of n-3 FA with 50% ALA (Moallem et al., 2012). However, the process of improving the quality of milk with n-3 FA through flaxseed supplementation also presents challenges, primarily due to ruminal biohydrogenation of ALA. Several studies have investigated n-3 transfer efficiency of different forms [extruded flaxseed (**EF**), ground flaxseed (**GF**), micronized flaxseed, and flaxseed oil] of flaxseed from the diet into the milk of dairy cows (Mustafa et al., 2003a; Moallem et al., 2012; Lerch et al., 2012b;

Ferlay et al., 2013; Resende et al., 2015; Isenberg et al., 2019) and observed a greater recovery rate of n-3 FA in milk compared to control diet.

Also, an EF-based supplement LinPRO-R (**LNPR**; O&T Farms Ltd., Regina, SK, Canada) was prepared combining flaxseed, alfalfa hay and field peas as described by Swanepoel and Robinson, (2019a) which has been shown a greater transfer efficiency of n-3 FA, particularly ALA into milk in confined dairy system compared to the diet with no flaxseed (Moats et al., 2018; Swanepoel and Robinson, 2019a; Swanepoel and Robinson, 2019b). However, little is known about the EF-based feed supplements on milk FA composition in pasture-based diet.

Energy is the most limiting nutrient in pasture-based diets (Bargo et al., 2002a; Hafla et al., 2016). Therefore, lipid supplementation has been a common strategy to increase the energy density of the diet fed to dairy cattle. The enthalpy of FA (9.3 Mcal/kg) is greater than that of starch (4.2 Mcal/kg) which can be used as a post-absorptive energy source and can directly transfer to the milk (NRC, 2001; Rico et al., 2014; Boerman et al., 2015). Feeding 10.5% (diet DM) LNPR did not have any effect on energy intake compared to a control diet (Judy et al., 2019). Furthermore, studies with feeding EF on dry matter intake (**DMI**), milk yield and components, N utilization, ruminal fermentation, milk FA profile, and nutrient digestibility of grazing dairy cows are scarce as well. Whereas feeding incremental amounts (0, 5, 10, and 15 % of the diet DM) of EF with hay or corn silage diet did not affect DMI and milk yield (Martin et al., 2016), a significant decrease in milk yield, DMI, and nutrient digestibility of dry matter (**DM**), organic matter (**OM**), acid detergent fiber (**ADF**), and neutral detergent fiber (**NDF**) were reported with feeding 14.8% (diet DM) EF (Martin et al., 2008). Contrarily, feeding LNPR at 0, 2.5 and 5% diet DM basis in a commercial dairy farm increased yield of milk fat, protein, and

lactose in addition to the greater apparent digestibility of OM, and NDF (Swanepoel and Robinson, 2019a).

It has also been shown that EF or flaxseed oil separately when added to the diet as a fat supplement decreased enteric methane (**CH_4**) emission in dairy cows (Martin et al., 2008; Beauchemin., 2009; Martin et al., 2016; Boland et al., 2020). Overall, studies where CH_4 production was reduced with feeding flaxseed observed a negative effect on DMI, and digestibility of nutrients (Martin et al., 2008; Beauchemin et al., 2009). It also appears that feeding different forms (ground vs extruded) of flaxseed respond differently to the daily CH_4 production (Martin et al., 2008; Almeida et al., 2015; Martin et al., 2016). Martin et al. (2016) showed linear reduction in CH_4 production, particularly CH_4 production decreased by 10.7% (across the 2 experiments with hay and corn silage diet) with feeding 5% EF compared to a control diet without flaxseed. It should also be noted that CH_4 production decreased more with hay than corn silage between control and 5% EF. Hence, the potential of flaxseed as fat supplement in reducing CH_4 emissions from dairy cows largely depends on the forms fed and the level of inclusion (% of DM) in the diet. However, studies with feeding EF on enteric CH_4 emissions in pasture based dairy system are limited.

Our objectives were 1) to evaluate an EF-based supplement LNPR (6% of the diet DM) versus a control diet (where the flaxseed was replaced with extruded and roasted soybeans) on production performance, and milk FA composition during grazing season; 2) to investigate whether 6% extruded flaxseed can lower enteric CH_4 emissions when accompanied by pasture and 3) to study how flaxseed as fat supplement contribute to the nutrient digestibility and the dynamics of energy utilization in grazing dairy cows. We hypothesized that LIN (LinPRO-R diet = **LIN**) would increase the energy density of the diet, thus increasing milk yield and components

compared to CTRL diet (Control diet = **CTRL**). Also, LNPR supplementation would increase nutrient digestibility of the LIN diet, suggesting more digestible energy (**DE**) will be partitioned to metabolizable energy (**ME**), and then increase the net energy for lactation ($\mathbf{NE_L}$). Lastly, CH_4 production might decrease due to the toxic effects of LNPR oil on ruminal methanogens.

MATERIALS AND METHODS

The care and management of the cows used in this study was conducted in accordance with the rules outlined by the University of New Hampshire Institutional Animal Care and Use Committee (IACUC Protocol no. 220602). This study was carried out at the University of New Hampshire Burley-Demeritt Organic Dairy Research Farm (Lee; 43°17′N, 70°93′W) between July 1 to September 22, 2022. Hourly environmental temperature (minimum = 14.2°C; maximum = 26.6°C; average = 20.3°C) and relative humidity (minimum = 45.2%; maximum = 89.8%; average = 69.8%) were obtained from a National Climate Data Center weather station installed at the University of New Hampshire Kingman Farm (Durham, NH) located approximately12 km away from the experimental site.

Cows, Experimental Design, and Treatments

Eighteen multiparous organic certified Jersey cows averaging (mean ± SD) 128 ± 52 days in milk (**DIM**), 26 ± 4.33 kg/d of milk, and 480 ± 33 kg of body weight (**BW**) and 2 primiparous organic certified Jersey cows averaging 128 ± 55 DIM, 19.6 ± 3.50 kg/d of milk, and 433 ± 23 kg of BW were used at the beginning of the study. Diets were formulated to meet or exceed the nutrient requirements of a lactating Jersey cow averaging 19 kg/d of DMI, and 24 kg/d of milk with 4.8 % milk fat and 3.3 % true protein using the NASEM (2021) ration evaluation software. Cows had access to a cool season grass-legume pasture mix overnight and received a partial

total-mixed ration (pTMR) during the day. The experiment was conducted during the grazing season and lasted a total of 12 weeks, with a 2-week covariate period before cows were assigned to treatments. During the covariate period cows had approximately 16 h access to the pasture and grazed cool season grass-legume pasture mix. Also, a pTMR diet was fed indoors and consisted of 34% forage and 66% concentrate. Mixed mostly grass baleage was used as forage source and corn meal and ground barley grain were the 2 major ingredients in the pTMR (Table 2). The pTMR averaged 16.1% crude protein (**CP**), 19% ADF, and 34.7% NDF. Milk, blood, ruminal fluid, BW, and body condition score (**BCS**) measurements were taken one week prior to the study. Dry matter intake data were also recorded. Covariate samples were analyzed according to the same procedure as described for weekly samples, and data were included in the statistical analysis for the variables mentioned above. The data and sample collection following animals assigned to the experimental diets occurred at wk 4 (August 5 to 11), wk 7 (August 26 to September 1), and wk 10 (September 16 to 22). Cows were adapted to the treatments for 3 weeks before samples collection started on wk 4. Cows were housed in a bedded pack barn with dried pine shavings as the bedding material all in the same pen separated from the other cows of the herd. Animals were trained to access the electronic recognition Calan gates (American Calan Inc., Northwood, NH) to individualize feed intake for approximately 2 wk before the beginning of the covariate period. The bedding area (132 m^2) opens to a concrete floor outdoor lot (478 m^2) in compliance with the USDA National Organic Program livestock living condition regulations (USDA AMS, 2010) that mandate year-round access to the outdoors for all ruminants. Milkings occurred at 0430 and 1530 h and milk yield were recorded individually throughout the study. A 4-stall step-up parlor equipped with headlocks (Agromatic; Fond Dulac, WI), automatic take-offs, and milk meters (Westfalia Surge; GEA Farm Technologies Inc., Naperville, IL) was used

for milking. Body weight (Northeast Scale Co., Inc., Hooksett, NH) and BCS measurements were recorded for 2 consecutive days after the p.m. milking during the last 2 d of the covariate and sampling periods. Body condition score was assessed from 1 to 5 scale by 3 individuals following Wildman et al. (1982).

Cows were blocked in pairs (n = 10) by DIM, milk yield, or parity and, within pair, randomly assigned to treatments in a randomized complete block design as follows: 1) grazed herbage plus pTMR (control diet = **CTRL**) or 2) grazed herbage plus pTMR and and LinPro-R (LinPRO-R diet = **LIN**). Cows assigned to CTRL averaged (mean ± SD) 117 ± 53 DIM, 25.7 ± 4.5 kg/d of milk, and 472 ± 25 kg of BW, while LIN cows averaged (mean ± SD) 139 ± 50 DIM, 25.8 ± 4.9 kg/d milk, 478 ± 44 kg of BW. LinPRO-R (O&T Farms Ltd., Regina, SK, Canada) is an extruded flaxseed-based energy supplement available commercially and designed to feed ruminants. According to the manufacturer, LinPro-R contains (DM basis) 55% extruded flaxseed, 38% field peas, and 7% alfalfa meal. Diets were formulated to yield (DM basis) approximately a 60:40 forage to concentrate ratio. A ground corn meal and barley grain-based concentrate mash was combined with a mixed, mostly legume baleage (alfalfa 70%, orchardgrass 14%, timothy 8%, tall fescue 7%, and red clover 1%) to produce the partial total mixed ration (**pTMR**) fed in the CTRL diet. A second concentrate mash formulated with lower concentrations of ground corn and soybean meal was mixed with the same legume-based baleage to make the pTMR offered in the LIN diet. LinPro-R (6% of the diet DM) was top-dressed to the pTMR in the LIN diet and mixed manually to ensure homogenous distribution of the supplement in the ration. The baleage used in the experimental diet was harvested and preserved in plastic wrapped bales as described by Resende et al. (2015). The ingredient composition of both pTMR and

concentrate mash are presented in Table 3. The nutrient and chemical composition of pTMR, concentrate mash, and the pellets are presented in Table 4.

Pasture and Feeding Management and Sample Collection and Analyses.

Cows were allowed to graze a cool season grass-legume herbage mix from 1730 h until 0430 h next morning before milking resulting in approximately 11 h of access to pasture daily following a strip grazing protocol described by Brito et al. (2017). To determine pasture biomass, herbage samples were collected above 3 cm from the ground level along 2 transect lines with 5 clippings each, totaling 10 clippings in each paddock using a hand shear and a quadrat measuring 0.25-m^2. Quadrats were thrown randomly following transect lines and representative samples were collected from the whole paddock area. If an area of the quadrat contained manure, it was thrown again. Total paddock area (m^2) was calculated using a global positioning system device (Garmin Ltd.). Fresh and dried (55°C in a forced air oven) weights of individual clipping in each transect were recorded. Total herbage biomass of the paddock was calculated by considering the average (n=10 clippings/paddock) dry weight of one clipping (0.25-m^2 area). Next, the whole paddock area was divided into 7 strips whereby each strip was allocated daily to grazing. Each strip was set up to provide an herbage allowance of at least 15 kg of DM/cow daily.

The determination of herbage biomass and the allocation of pasture was done every week across the study. During each sampling week, pre-grazing herbage biomass was determined by collecting 20 herbage samples along with 2 transect lines (10 clippings/transect) on d 1, and d 4 following the same procedure described above. To determine the post-grazing biomass, 30 herbage samples along with 3 transect lines (10 clippings/transect) were collected on d 3, d 5, and d 7 during each sampling week. The individual weight of the fresh and dried sample in each clipping was recorded as described above and then averaged over the total clippings to determine

the pre- and post-grazing biomass of the entire paddock. A slightly different methodology was used for post-grazing sample collection (30 samples on 3 different days following 3 transect lines) which was due to potential pasture regrowth of the paddock after grazing so that the error associated with post-grazing biomass calculation can be minimized. Next, the herbage disappearance was calculated by subtracting post-grazing biomass from pre-grazing biomass yield. Pre- and post-grazing herbage height measurements (n=15 heights/paddock) were also taken using a ruler along with 3 transect lines (5 heights/transect) in each sampling week.

Samples for determination of botanical composition (n=15) were collected on d 1 from the whole paddock along with 3 transect (5 samples/transect) following the same protocol as described by Isenberg et al. (2019). After collection, samples were sorted into grasses, legumes, weeds, and dead material. Later, individual herbage samples were dried in a forced air oven for 48 h at 55°C and then averaged over 15 samples to determine herbage botanical composition (%) of the whole paddock. To assess herbage nutritional composition, pasture samples were collected (n=20) on d 1 and d 5 using hand plucking method (Kolver and Muller, 1998) followed by 2 transect lines (10 clippings/transect) throughout all paddocks set to be grazed by experimental cows. Collected samples were stored at 4°C until further processing. Individual samples were dried in a forced air oven for 48 h at 55°C. Dried samples were stored in a container at room temperature until grinding. Afterwards, the samples were ground to pass through a 1-mm screen (Wiley mill; Thomas Scientific) and composited into 2 samples per sampling week (1 sample/transect) and shipped to Dairy One Forage Laboratory (Ithaca, NY) for chemical analyses.

The pTMR fed in the present study was prepared by mixing the individual ingredients using a Jaylor A100 Self-Propelled mixer (Jaylor Fabricating Inc., East Garafraxa, ON, Canada)

and offered once daily at 0530 h in the Calan gates after the morning milking. Cows had free access to drinking water in the barn and pasture throughout the duration of the experiment, and animals were fed the pTMR to yield a maximum of 5% orts. All bales used in the study were sampled (~150 g of baleage/bale) prior to the beginning of the experiment using an electric drill (model TE 7-A; Hilti Inc.) fitted with a 45-cm stainless steel core sampler barrel. Samples were dried at 55°C in a forced air oven for 48 h and used to adjust the proportion of baleage in the pTMR daily. Concentrate mash and LIN were dried as done for baleage to adjust their proportions in the pTMR. Water was added to the pTMR to achieve about 65% DM, which was confirmed by weekly collections of pTMR samples for DM analysis.

Chromium sesquioxide (City Chemicals LLC., West Haven, CT) was incorporated into a pelleted feed by Morrison's Custom Feeds Inc. (Barnet, VT) to estimate the fecal output of DM. The target chromium oxide ($\mathbf{Cr_2O_3}$) was set at 10 g/d (Bargo et al., 2002a) and was accomplished with feeding (DM basis) 834 g of the custom-made pellet daily (split in 2 feedings) into during the last 10 d of each sampling period. A proportional amount of the concentrate mash was substituted, based on dry matter, with 834 g of a custom-made Cr_2O_3 pellet, which had similar ingredient composition to the concentrate mash. Pelletized Cr_2O_3 was placed in rubber pans inside the calan door of each cow before morning feeding at 0500 h and after afternoon milking at 1600 h. Total consumption of the pellets was confirmed visually.

The pTMR and orts samples (~ 400 g) from both diets were collected daily during each sampling week, stored in zip-loc bags at 4°C, and later dried in a forced air oven (55°C, 48 h) for DM determination and calculation of pTMR DMI. Concentrate mash, LNPR, and pellets were sampled once per sampling week and stored at 4°C until further processing. Samples of baleage (~200 g) were also collected daily immediately after chopping a new baleage during each

sampling week. The pTMR and orts samples collected across the sampling week were composited. All feeds were dried (55°C, 48 h), ground to pass through a 1-mm screen (Wiley mill; Thomas Scientific).

Ground feed samples were sent to the Dairy One Forage Laboratory (Ithaca, NY) for wet chemistry analysis according to the following methods: absolute DM (method 930.15; AOAC, 2016), CP (total N × 6.25; method 990.03; AOAC, 2016), soluble protein (Cornell Sodium Borate-Sodium Phosphate Buffer procedure; Cornell Nutrition Conference Proceedings, 1990), acid detergent insoluble crude protein (**ADICP**) and neutral detergent insoluble crude protein (**NDICP**) (Leco TruMac N Macro Determinator; Leco Corporation, St, Joseph, MI), ADF [method 5, Ankom Technology; solutions as in method 973.18 (AOAC, 2016)], ash free neutral detergent fiber organic matter (**aNDFom**) [method 6, Ankom Technology; as in Van Soest et al. (1991) residues incinerated at 550°C for 2 h], starch (YSI 2700 Select Biochemistry Analyzer, application note no. 319; YSI Inc. Life Sciences, Yellow Springs, OH), ether extract (**EE**) [extraction by a Soxtec HT6 System (Foss North America) using anhydrous diethyl ether; method 2003.05 (AOAC, 2016)], ash (method 942.05; AOAC, 2016), individual minerals: Ca, P, Mg, K, Na, S, Fe, Zn, Cu, Mn, Mo (Thermo IRIS Advantage HX or ICAP 6300 intrepid inductively coupled plasma radial spectrometer after microwave digestion (CEM application note for acid digestion; CEM, Matthews, NC), chloriode ion (Brinkmann Metrohm 716 Titrino Titration Unit with silver electrode (Metrohm application bulletin no. 130; Metrohm Ltd., Herisau, Switzerland) as described by Dairy One Forage Testing Laboratory (https://dairyone.com/download/forage-forage-lab-analytical-procedures/?wpdmdl). Ground Cr_2O_3-pellet from each sampling period were also shipped to Analab (Fulton, IL) to measure chromium (**Cr**) concentration using atomic absorption (Williams et al., 1962; Binnerts et al.,

1968). In vitro true dry matter digestibility (**IVTDMD**; 30 h ruminal digestibility using live artificial rumen, and 24 h enzymatic digestion) for forage and concentrates samples was done by AnaLab (Fulton, IL). In-vitro dry matter digestibility (**IVDMD**) of all the forages and concentrates was also determined for 24 h and 48 h by Cumberland Valley Analytical Service (Waynesboro, PA) using a Daisy II incubator (AN-KOM Technology, Fairport, NY). Composite feed samples were also sent to the Pennsylvania State University (Kevin Harvatine Laboratory, University Park, PA) for FA analyses by gas chromatography after direct methylation as described by Sukhija and Palmquist, (1988). Gross energy (**GE**) of all composited feeds, urine, and feces was measured using an oxygen bomb calorimeter Parr 6200 (Parr Instrument Company, Moline, IL). The gross energy of all the feed samples was measured using a parr 6200 oxygen bomb calorimeter (Parr Instrument Company, Moline, IL).

Milk Sampling and Analyses

Milk yield was recorded during every morning (0430 h) and afternoon (1530 h) milkings throughout the duration of the experiment. Milk samples were collected during 4 consecutive milkings starting in the afternoon milking of d 1 and finishing in the morning milking of d 3 of each sampling period using automatic samplers. Following collection, samples were transferred to vials containing 2-bromo-2- nitropropane-1,3 diol tablet (Broad Spectrum Microtabs II; Advanced Instruments Inc., Norwood, MA) and shipped to Dairy One DHIA (Ithaca, NY) for analyses of milk fat, true protein, lactose, solids-not-fat (**SNF**), total solids (**TS**), and milk urea nitrogen (**MUN**) by Fourier-transform infrared spectroscopy using a MilkoScan FT+ (Foss Inc., Hillerød, Denmark), and somatic cell count (**SCC**) by flow cytometry with a Fossomatic FC (Foss Inc.). Milk samples collected without preservative were composited based on the milk yield of the afternoon milking of d 1 and that of the morning milking of d 2 of each sampling

period and stored at -80°C until FA analyses at the Pennsylvania State University using GLC following the method reported by Rico and Harvatine (2013). Yields of energy corrected milk (**ECM**) and 4% fat corrected milk (**FCM**) were calculated according to Orth (1992) and Gaines and Davidson (1923), respectively.

Blood Sampling and Analyses

Blood was sampled from the coccygeal vessels by venipuncture on d 5 of each sampling period approximately 5 h after the morning feeding into 15% EDTA vacutainer tubes (Covidien Monoject). Samples were kept in a chill bucket with beads immediately after collection. Within 1 h of collection, samples were centrifuged (Eppendorf model 5810) at 3,300 × g for 20 min at 4°C. Plasma samples were pipetted into 1 mL test tube and were stored at -20°C for later analysis of plasma urea N (**PUN**) using a commercial kit from Stanbio Laboratory (Catalog No. 10152-590), and absorbance read at 540 nm with a UV/ visible spectrophotometer (Beckman Coulter Inc., Brea, CA).

Ruminal Fluid Sampling and Analyses

Ruminal fluid samples were collected on d 7 of each sampling period approximately 5 h after the morning feeding. Cows were restrained in headlocks, and samples were taken using a stomach tube apparatus (polytube attached to a plastic Erlenmeyer flask and a vacuum pump). The Initial 200–300 mL of sample was discarded to minimize saliva contamination. Immediately after collection, samples were transferred to 400 mL beakers and filtered through 4 layers of cheesecloth. After filtration, 10 mL aliquots of ruminal fluid were pipetted into 15 mL tube containing 0.2 mL of 50% (vol/vol) H_2SO_4. Samples were placed in dry ice immediately after collection and later stored at -20°C until volatile fatty acids (**VFA**) analysis. Ruminal fluid

samples were sent to Dairy One Forage Laboratory for VFA analysis following water extraction method using a Perkin Elmer Clarus 680 Gas Chromatograph containing a Supelco packed column with the following specifications: 2 m × 2 mm Tightspec ID and 4% Carbowax 20M phase on 80/120 Carbopack B-DA.

Urinary and Fecal Sampling and Analyses

Spot urinary samples were collected 5 times across 3 consecutive days during each sampling period at 0900 and 1500 h on d 2, 0600 h on d 3, and 1200 and 1700 h on d 4 by stimulating pudendal nerve below the vulva. Cows were restrained in headlocks during urine collection. Samples were collected into 60 mL cups and composited by cow over 3 d by pipetting 8 mL of urine into a tube containing 32 mL of 0.072 *N* H_2SO_4 yielding a total volume of 40 mL. All composited urine samples were stored at -20°C until further analyses. After thawing at room temperature, acidified urine samples were analyzed for total N, urea N, creatinine, and purine derivatives (**PD**; allantoin and uric acid). To determine CP, 10 mL acidified urine samples were sent to Dairy One Forage Laboratory using a Leco TruMac N Macro Determinator (Leco Corporation, St. Joseph, MI) using the combustion method (AOAC 990.03, 1990). Urinary creatinine was performed using a commercially available kit (Catalog no. 500701; Cayman Chemical Company), and chromate microplate reader (Awareness Technology, Inc., Palm City, FL) at 492 nm wavelengths using a UV/visible spectrophotometer. Quantitative determination of uric acid was done using the uric acid assay kit (Catalog no. DIUA-250; BioAssay systems, Hayward, CA) at 590 nm wavelengths. Urinary urea N was determined at 522 nm wavelength using a urea N test kit by diacetylmonoxime method (Catalog no. 0580-250; Stanbio Laboratory). Urinary allantoin assays were performed using a microplate reader at 530 nm wavelengths by phenylhydrazine determination method described by Chen and Gomes (1992).

Total urinary volume was calculated based on the estimated creatinine (mmol/d) excretion which was assumed 0.212 mmol/kg of BW (Chizzotti et al., 2008). Total PD (mmol/d) were determined by adding the excretion of allantoin (mmol/d) and uric acid (mmol/d) as described by Chizzotti et al. (2008). The GE of urine was determined by using 4 g of acidified urine samples in combustion capsule and dried in the oven for 12 h at 55°. The dried samples were then set for combustion using using parr 6200 oxygen bomb calorimeter (Parr Instrument Company, Moline, IL). Later, the GE of urine samples was adjusted based on the dilution of acid and urine.

Fecal grab samples were collected 8 times (0600, 0900, 1100, 1200, 1300, 1500, 1600, and 1700 h) over 5 d starting on d 2 of each sampling period and finishing on d 6. Samples were pooled by cow and dried in a forced air oven at 55°C for approximately 96 h. Dried samples were ground to pass through a 1-mm screen (Wiley mill) and shipped to Dairy One Forage Laboratory to be analyzed for absolute DM, CP, aNDFom, ADF, and ash according to methods reported above. Fecal samples were also sent to Analab for Cr analysis using the same procedure described above. Fecal output (kg DM/d) of each cow was estimated by Cr intake (g/d/cow) and fecal excretion of Cr (g/d/cow) as described by Kolver and Muller (1998) using the following equation:

Fecal output of DM (kg/d) = Cr intake (g/d)/fecal Cr output (g/kg of DM)

Herbage DMI (kg/d) was estimated as described by Bargo et al. (2002a) as follows:

Estimated herbage DMI (kg/d) = [{fecal DM output (kg/d) – GreenFeed pellet DMI (kg/d) x (1 - IVDMD of GreenFeed pellet) – Cr_2O_3 pellet DMI (kg/d) x (1 - IVDMD of Cr_2O_3 pellet) - TMR DMI (kg/d) x (1- IVDMD of TMR)] / (1 - IVDMD of pasture)

Also, feces samples (0.4 g) were weighed and set for combustion using a parr 6200 oxygen bomb calorimeter (Parr Instrument Company, Moline, IL) to measure the DE.

Gaseous Measurements and Energy Utilization Calculations

Two portable automated gas quantification units [GreenFeed (**GFeed**); C-Lock Inc., Rapid City, SD] were used to measure gaseous emissions (CH_4 and CO_2) throughout the experiment. One GFeed unit was used in the barn, and the second one set up for pasture measurements was maintained in the paddock and kept at the closest proximity of the cows as possible. Cows had free access to both GFeed units except during milkings, with cows detected by the GFeed system through their radio frequency identification ear tags. The pasture GFeed unit was equipped with an automatic gaseous (zero and span) calibration system, while gaseous calibration for the GFeed unit used in the barn was done manually every week. A CO_2 recovery test was done for both GFeed units on the last week of every month. All calibration protocols and maintenance were done according to the manufacturer's recommendations (https://globalresearchalliance.org/wp-content/uploads/2018/08/GreenFeeds-SOP-_final.pdf). A dairy pellet (Morrison's MP 14% CP pellet; Morrison's Custom Feeds Inc., Barnet, VT) was used as bait to attract cows to GFeed units. The feeding schedule for each GFeed unit was set at 4 visits/d with 8 cup drops/visit at 15 s interval between drops spaced by 3.5 h from one visit to the next. The GFeed units were set to dispense a maximum of 34 g of pellet per drop. Pellet intake was included in the total DMI calculations. Gaseous emission data for the last 10 d of each experimental period were used to calculate CO_2 and CH_4 production (g/d) CH_4 yield (g/kg of DMI), and CH_4 intensity (g/kg of ECM). The equations used to calculate the intake of energy and energy utilization efficiency of cows have been listed below:

DE intake (Mcal/d) = GE intake (Mcal/d) – fecal energy (Mcal/d).

ME intake (Mcal/d) = DE intake (Mcal/d) – urinary energy (Mcal/d) – CH_4 energy (Mcal/d).

NE_L intake (Mcal/d) = ME intake (Mcal/d) × 0.66 (NASEM, 2021)

CH_4 energy (Mcal/d) = CH_4 production (g/d) × CH_4 enthalpy (9.45 Kcal/L)

Heat Production (**HP**; Mcal/d) = 3.866 × O2 (L/d) + 1.200 × CO2 (L/d) – 0.518 × CH4 (L/d) – 1.431 × urinary N excretion (g/d) (Brouwer, 1965)

Milk energy (Mcal/d) = [(0.0929 × milk fat%) + (0.0585 × milk true protein%) + (0.0395 × milk lactose%)] × milk yield [(kg/d; NASEM, 2021)]

Tissue energy (Mcal/d) = ME intake (Mcal/d) – heat production (Mcal/d) – milk energy (Mcal/d).

Statistical Analyses

All data were analyzed using MIXED procedure of SAS (release 9.4, SAS Institute Inc.) according to a randomized complete block design with repeated measure over time. The following model was used:

$$Y_{ijk} = \mu + B_{i+} T_j + W_k + ßC_{ijk} + T\times W_{jk} + \varepsilon_{ijkl}$$

where Y_{ijk} = dependent variable, μ = overall mean, B_i = random effect of the ith block (pair of cows), T_j = fixed effect of jth treatment, W_k = fixed effect of kth week, ß = regression coefficient of the covariate C, C_{ijkl} = the covariate variable for the lth cow within the ith block of the jth treatment in the kth week, $T \times W_{jk}$ = the interaction between the jth treatment and the kth week, and ε_{ijkl} = error term (assumed to be normally distributed with mean = 0 and constant variance). Intake, apparent total tract digestibility of nutrients, energy partitioning, and gaseous emissions data were analyzed without adjusting covariate in the model. Data were tested for normality using the UNIVARIATE procedure of SAS, and outliers were identified and removed from the

statistical analyses if studentized residuals were >3.0 or <−3.0. Log transformation (i.e., SCC) was done when W statistics of Shapiro-wilk is less than 0.05. The SAS command REPEATED was used to model distinct residual variances, and among the covariance structures tested [i.e., spatial power, compound symmetry, autoregressive (1), and heterogeneous autoregressive (1)], the one with the least Bayesian information criterion was retained in the final model. The subject of the repeated measures was defined as cow nested within treatment and was treated as a random effect in the model. All reported values are presented as LSM ± SEM, unless otherwise noted. Least square means were separated by pairwise t-test using the PDIFF option of the MIXED procedure of SAS if $P \leq 0.05$. Furthermore, least square means within sampling week (i.e., wk 4, 7 and 10) were partitioned with the SLICE command of SAS and separated pairwise t-test when diet × week interactions were $P \leq 0.05$. The selection of whether to use full or reduced models for variables with covariates or interaction terms, or both, with $P > 0.25$ was determined by comparing the Bayesian information criterion values of the models and selecting the model (full or reduced) with the lowest value. Significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$. The interaction term was removed from the model when $P > 0.25$.

RESULTS AND DISCUSSION

Nutritional composition of Herbage and Feeds

The ingredient composition of LNPR is listed in Table 1. The ingredient composition of both CTRL and LIN diet are presented in Table 2. The ingredient composition of concentrates mash and pellets are listed in Table 3. The nutrient composition of the baleage, concentrate mash, LNPR, both CTRL and LIN pTMR, and pellets are shown in Table 4. The FA composition of LNPR is listed in Table 5. The NASEM (2021) evaluation of the experimental diets are presented in Table 6. Environmental conditions, herbage biomass and allowance, sward botanical composition, and herbage nutritional composition are presented in Table 7. Herbage mineral composition and FA profile are shown in Table 8. Pre-grazing herbage mass averaged 2,048 kg of DM/ha and ranged from 1,362 to 2,563 kg of DM across the grazing season. Daily herbage allowance averaged 14.9 kg of DM per cow resulting from a mean value of 78 m^2 of pasture area provided per cow/d. The highest pasture area was provided during wk 10 to compensate for the lowest pre-grazing biomass and to maintain herbage allowance similar throughout the experiment. It should be noted that herbage allowance offered in this study was 123% greater than the estimated herbage DMI (mean = 6.67 kg/d; Table 9), thus indicating that the amount of herbage available did not restrict herbage DMI.

Grasses were the predominant forage species in sampled herbage averaging (DM basis) 49.6%, with legumes and weeds averaging 14.5 and 15.0%, (Table 7). Brito et al. (2017) reported a similar botanical composition pattern in herbage samples collected throughout the grazing season. The proportion of grasses observed in the current study was 3.4-fold greater than that of legumes, thus in line with previous grazing studies conducted at the same location, which averaged 50.6 and 15.2% for grasses and legumes, respectively (Brito et al., 2017; Isenberg et

al., 2019). The CP concentration of herbage sample was lowest in wk 4 (12.0%) and increased thereafter in wk 7 (18.4%) and 10 (22.0%) as shown in Table 7 but the NDF and ADF concentrations remain unchanged in wk 4 and 7, and increased in wk 10, which may be associated with botanical composition, particularly due to the variation of species composition in grass and legumes, or plant growth stages that can significantly affect herbage nutrient composition. The increased CP concentration in wk 7 compared to wk 4 could also be due to the greater proportion of weeds that was observed in wk 7, and it is possible that some weed species may contain high CP concentration and more fiber.

The ALA was the predominant (49 g/100 g of FA; Table 8) FA found in herbage samples which agrees with previous studies (Brito et al., 2017; Hafla et al., 2018; Isenberg et al., 2019). The second and third most prevalent FA in the herbage sample was *cis*-9, *cis*-12 18:2 and 16:0, respectively. The proportion of ALA in herbage samples was numerically greater (mean = 58.8 g/100 g of FA) in wk 10, followed by wk 7 (mean = 49 g/100 g of FA) and wk 4 (mean = 39 g/100 g of FA). This variation of ALA may be due to the seasonality, changes in plant botanical composition, or plant growth stage (Boufaïed et al., 2003; Mir et al., 2006). As expected, ALA is the main FA present in LNPR, which averaged 46.2 g/100 g of FA (Table 5) followed by *cis*-9 18:1 (mean = 20.4 g/100 g of FA). A similar concentration of ALA (i.e., 46.3 g/100 g of FA) in LNPR was reported by Moats et al. (2018).

The CON concentrate mash, and the LIN concentrate mash were included in the CTRL and LIN diet respectively. The LIN concentrate mash was formulated to yield lower CP compared to the CON concentrate mash and fed lower amount (% of diet DM), but 6% LNPR (of diet DM) was used to compensate for this difference in the diet. Both diets yielded similar

levels of CP (Table 6), but the NE_L intake was slightly higher in the LIN diet than CTRL (1.77 vs. 1.70 Mcal/kg of DM) due to the inclusion of LNPR.

Feed intake, Body Weight, and Milk Yield and Composition

Dry matter intake (**DMI**), milk yield and composition, BW, and BCS are presented in Table 9. A greater (P = 0.01) estimated herbage DMI was observed for CTRL than LIN cows (7.39 vs 5.95 kg/d). A treatment × week interaction was also found for estimated herbage DMI which was greater in week 4 and 7 with CTRL diet compared to LIN. Estimated herbage DMI (mean = 6.67 kg/d) was, on average, 29% of the total DMI (mean = 22.8 kg/d) in the current study, which was 11 percentages units lower than the formulated target of 40% in the diet DM. Our previous study using 0 or 10% GF in grazing cows also reported a lower herbage DMI (34%) than the formulated target values (40%; Isenberg et al., 2019). However, the estimation of herbage DMI has been difficult in the past following different external (i.e., Cr_2O_3, titanium oxide) and internal markers [i.e., indigestible neutral detergent fiber (**iNDF**), acid detergent lignin (**ADL**) of the feedstuffs] (Hellwing et al., 2015). The herbage DMI offered herein was estimated by Cr_2O_3 as an external marker and using a combination of 24-h and 48-h IVDMD of dietary ingredients according to procedures reported by Bargo et al. (2002a). Although Titgemeyer (1997) observed an average of 94% fecal recovery rate of Cr in 9 studies using Cr_2O_3 as external marker, Smith and Reid (1954) reported an intraday variation of Cr excretion in the feces when fecal samples were collected for 7 days at different intervals (2h intervals on day 2 and day 6^{th}, and 6 h interval for the rest of 5 days). However, in the next experiments when fecal samples were collected at 6 a.m. and 4 p.m. over 7 days, the authors were able to get a reliable fecal output using Cr intake and fecal Cr excretion data (Smith and Reid, 1954). In the current study, fecal samples were collected 8 times over 5 days, suggesting less variation of fecal Cr

excretion as explained by Smith and Reid (1954). Therefore, this difference in herbage DMI between CTRL and LIN cows could not be explained by the diurnal variation of fecal Cr excretion. Rather, substitution effect could be the reason that may have resulted in lower herbage intake in LIN cows because total supplement DMI tended to be greater for LIN cows compared to CTRL. In studies, where the herbage DMI was 3.5 kg/d lower when cows were fed 0.5 kg/d hydrogenated oil as fat supplement compared to control showed energy homeostatic mechanisms as plausible causes that regulated herbage DMI (Schroeder et al., 2002); however, the energy intake was similar between the treatments. Some other studies also reported metabolic regulation of DMI after feeding protected fat supplements (Choi and Palmquist, 1996; Rodriguez et al., 1997). Overall, the substitution effect, metabolic control, or even negative effects of LNPR supplements could be the causes for lower herbage DMI in LIN cows compared to CTRL.

No difference was observed in pTMR intake between the diets that averaged 14.7 kg/d. A tendency ($P = 0.07$) was observed for total supplement DMI to increase in CTRL than LIN cows (16.3 vs. 15.9 kg/d). Likewise, total DMI tended ($P = 0.06$) to be greater in CTRL compared to LIN (23.3 vs. 22.3 kg/d) due to increased herbage DMI with feeding CTRL (Table 9). Consistent with this finding, it was observed that feeding 21.2% EF product (70% EF and 30% wheat) of diet DM to the dairy cows resulted in a decrease in DMI (Martin et al., 2008). Similarly, Moats et al. (2008) also observed a 7% decrease in total DMI when dairy cows were fed 11.4% EF of diet DM compared to a CTRL diet. Lerch et al. (2012b) fed a CTRL diet, EF, and different forms of rapeseed (to provide 2.5-3.0% oil of diet DM) in a grass-based diet and found a decrease in DMI with EF diet compared to CTRL. Contrarily, others reported no effect on total DMI when supplemented EF at 10.5% of the diet DM (Judy et al., 2019) or at incremental levels (0, 5, 10, 15% diet DM) with either hay or corn silage diet (Ferlay et al., 2013). The latest experiment

conducted feeding 0, 2.5, and 5% (diet DM) EF showed no effect on total DMI (Swanepoel and Robinson, 2019b). However, it appears that the decrease in total DMI for LIN was due to the lower (P = <0.01; -1.44 kg/d) herbage intake compared to the CTRL cows because pTMR intake fed indoor was similar between the diets. Some other reasons for instance, release of gut hormones, and oxidation of FA in the liver could also be associated with the negative effects of fat supplement on feed intake (Chilliard, 1993; Allen, 2000).

Milk yield (mean = 27.3 kg/d), 4% FCM (mean = 29.9 kg/d), and ECM (mean = 32.1 kg/d) did not differ between diets (Table 9). Feed efficiency expressed as ECM/total DMI or 4% FCM/total DMI increased (P = 0.05; 1.47 vs. 1.37) or tended (P = 0.06; 1.37 vs. 1.27) to increase in LIN than CTRL due to the decreased total DMI in LIN cows because no difference was found for 4% FCM yield and ECM across treatments. Moats et al. (2018) also reported a greater milk yield, and feed efficiency (ECM/total DMI) when cows were fed 11.4% (diet DM) EF compared to a CTRL diet. However, cows fed incremental amounts of EF (0, 5, 10, and 15% of the diet DM) either with hay or corn silage reported no difference in feed efficiency (fat and protein-corrected milk/DMI; Martin et al., 2016). Judy et al. (2019) fed LNPR at 10.5% of the diet DM and reported no changes in milk yield, 4% FCM, ECM, and feed efficiency. Likewise, Swanepoel and Robinson (2019b) did not find any effect on milk yield with feeding incremental levels of LNPR (0, 2.5, and 5% of the diet DM) from mid-to-late lactation dairy cows on a commercial farm. Conversely, the same authors observed a linear increase in milk yield when fed similar inclusion of LNPR in the diet to early lactation dairy cows (Swanepoel and Robinson, 2019a). Apparently, it was transparent from these 2 experiments that feeding LNPR to the early lactation dairy cows may be more beneficial than late lactation (Swanepoel and Robinson, 2019a; Swanepoel and Robinson, 2019b) due to the higher energy requirement during early

lactation than mid or late lactation which was also consistent with the findings from Petit (2010) who reported that positive milk response to feeding flaxseed was more common in early lactating cows. In the present experiment, LNPR was fed to the mid-to -late lactation dairy cows (139 ± 50 DIM) which may be a reason for the lack of response in milk yield and components. Indeed, feed intake has been shown to have a positive relationship with milk yield (NRC, 2001), and is widely considered as the single most important factor affecting production performance (Huhtanen et al., 2011). In the current study, DMI was greater in CTRL cows which did not necessarily increase milk yield, 4% FCM or ECM compared to LIN. In fact, the cows fed LIN diet produced similar amounts of milk even with lower DMI.

Neither milk fat concentration (mean = 4.66%) nor yield (mean = 1.26 kg/d) were different between treatments. Similarly, there were no treatment differences for milk lactose concentration (mean = 4.79%) and yield (mean = 1.32 kg/d), and milk true protein concentration (mean = 3.48%) and yield (mean = 0.95 kg/d). Feeding CTRL versus LIN also did not have any effects on other milk components (i.e., milk SNF%, milk SNF yield, milk TS%, milk TS yield) as well as SCC. Similar results were observed by Judy et al. (2019) with feeding LNPR at 10.5% diet DM. Except milk fat and protein concentration that decreased linearly and quadratically, respectively, Ferlay et al. (2013) reported no other changes in milk components and yield when cows were fed incremental amounts (0, 5, 10, 15% diet DM) of EF with hay-based diet. Swanepoel and Robinson, (2019a) observed no effect on milk fat and true protein concentration but greater yield of milk fat, protein, and lactose with feeding 0, 2.5, and 5% (diet DM) LNPR to the early lactating dairy cows. However, feeding the similar inclusion level LNPR to the mid-to-late lactation dairy cows increased milk fat concentration linearly, whereas lactose quadratically tended to be greater with no effect on milk yield and yield of other milk components (i.e., fat,

true protein, lactose; Swanepoel and Robinson, 2019b).The lack of response in the current study is also in agreement with Martin et al. (2008) who reported no changes in milk components (i.e., protein, and lactose %) except milk fat% which decreased with feeding 21.2% EF product (70% EF and 30% wheat) compared to CTRL. Moats et al. (2018) observed a higher lactose concentration whereas milk fat concentrations decreased, and the yield of milk fat was unaffected when the dairy cows were fed EF versus a CTRL diet. In the current experiment, the LNPR was added only 6% of diet DM which may not be great enough to induce milk fat depression as found in other studies with more than 10% EF (diet DM). These data from the current study are also consistent with our previous research in grazing dairy cows consuming 0 or 10% GF, where Isenberg et al. (2019) observed no difference in milk components.

Concentrations of MUN (8.38 vs 11.04 mg/dL) was lower (P <0.001) in LIN versus CTRL diet whereas no effect was observed for PUN which averaged 21.3 mg/dL. When ruminally accessible N is supplied, ammonia is produced in the rumen as a byproduct of ruminal fermentation which is readily available for microbial protein synthesis. Ammonia is then transported to the liver combined with carbon dioxide (**CO_2**) to form urea. Urea that has been synthesized in the liver enters in the bloodstream and equilibrates with the other fluids in the body including milk reflecting close relationship between PUN and MUN (Broderick and Clayton, 1997). The discrepant results between PUN and MUN observed in the current study are difficult to reconcile. Excessive dietary concentration of protein or N intake is a well-known contributor to the elevated levels of urea N in the body. In the current study, N intake (607 vs. 570 g/d) was greater (P <0.05) in CTRL compared to LIN, thus in line with increased MUN in cows fed CTRL diet. In a recent study, Souza et al. (2021) investigated PUN and MUN variations in lactating cows following gastrointestinal and kidney urea clearance rates with

feeding a common diet and reported that gut urea transport activity was different among cows which resulted in variation between PUN and MUN concentration. Therefore, it appears that gut urea transport activity of different cows could also be the reason for this large variation between PUN and MUN observed in the current study. Also, the MUN estimated in the current study from the composited milk samples that were collected from 4 different time points over 2 d, whereas the PUN was determined from the blood samples that were collected once at 1000 h. This sampling schedule captured a segment of the diurnal feeding cycle for PUN while encompassing the entire feeding cycle for MUN. This discrepancy in sampling intervals may also have contributed to the observed variations between MUN and PUN concentration.

Treatments had no effects on BW (mean = 475 kg), BW change (mean = -0.20 kg/d), BCS (mean = 2.53), and BCS change (~ 0.00) which agrees with Martin et al. (2016) and Swanepoel and Robinson (2019a) who fed incremental amounts (0, 5, 10, and 15% diet DM) of EF with hay or corn silage-based diet or 0, 2.5, and 5% (diet DM) LNPR, respectively.

Milk FA Profile

Effects of treatments on milk SFA are presented in Table 10. While the proportion of *iso*-17:0 FA increased ($P < 0.01$) out of total 15 SFA that were changed, every other SFA decreased ($P < 0.05$) in cows fed 6% LIN compared to CTRL. Similarly, except ∑ odd chain SFA, which tended ($P = 0.08$) to decrease in LIN versus CTRL diet, ∑ branched chain FA, ∑<16C FA, and ∑16C FA decreased ($P <0.05$) with feeding LIN. A greater proportion of ∑18C FA (36.0 vs 34.2 g/100 g of FA) was observed in LIN compared to CTRL, which corroborates a negative correlation between mammary gland de novo synthesis of FA and dietary intake of PUFA (Chillard et al., 2000). Consistent effects on the milk FA profile were reported in the literature when fed EF to the lactating dairy cows. Several investigations have reported a decrease of

∑<16C and an increase of ∑18C FA in milk when dairy cows were supplemented with 11.4% (diet DM) LNPR (Moats et al., 2018) or EF (to provide 2.5-3.0% oil of diet DM) in grass-based diets (Lerch et al., 2012b). Similar responses were observed in the previous studies when 10% GF (diet DM) were fed to grazing dairy cows (Isenberg et al., 2019). The elevated concentrations of Σ18:0 FA in milk of LIN cows were probably due to the greater concentration of 18:1, 18:2, and 18:3 FA in LNPR.

It is well established that the odd-chain and branched-chain FA are synthesized primarily from ruminal microbes (Vleminck et al., 2006). Therefore, it is possible that the inclusion of EF could have inhibited some of the ruminal microorganisms that are responsible for synthesis of odd- and branched-chain FA. In line with the result of the current study, the odd- and branched chain FA was also reduced in our previous experiment with feeding 10% GF to the grazing dairy cows (Isenberg et al., 2019). The proportion of Σn-6 and Σn-6/Σn-3 ratio of milk FA decreased with feeding LIN diet which is corroborated by others when cows were fed EF (Ferlay et al., 2013; Lerch et al., 2012b) or LNPR (Moats et al., 2013; Swanepoel and Robinson, 2019a, b).

Treatment effects on UFA are presented in Table 11. A total of 23 UFA was changed by treatments whereby 18 FA increased ($P < 0.05$), 5 FA decreased ($P < 0.01$) with feeding LIN. Whereas feeding LIN increased ($P < 0.05$) the proportion of ∑n-3, *cis*-11 18:1, *cis*-12 18:1, *trans*-9 18:1, and *trans*-11 18:1, the proportion of *cis*-9, *cis*-12 18:2, ∑n-6 FA, and n-6/n-3 ratio decreased. Also, the proportion of *cis*-9, *cis*-12, *cis*-15 18:3 (ALA; 0.93 vs. 0.59 g/100 g of FA) and *cis*-9, *trans*-11 18:2 (CLA; 0.62 vs. 0.53 g/100 g of FA) increased by 58% and 17% respectively, with feeding LIN versus CTRL. Results from the current experiment on the proportion of ALA and *cis*-9, *trans*-11 18:2 CLA are consistent with others when early or late lactation cows were fed LNPR at incremental levels (0, 2.5, and 5% diet DM); Swanepoel and

Robinson, 2019a,b), EF (to provide 2.5-3.0% oil of diet DM; Lerch et al. 2012b) or 11.4% LNPR compared to a CTRL diet (Moats et al., 2018). Similarly, feeding EF at incremental levels (0, 5, 10, and 15% diet DM) with hay or corn silage-based diet linearly increased milk ALA and CLA concentrations (Ferlay et al., 2013). A previous grazing study also reported an 89% increase in milk ALA with feeding 10% GF (Isenberg et al., 2019), which also agrees with Resende et al. (2015) where linear increase of ALA in milk of dairy cows were observed when fed at incremental levels (0, 5, 10, and 15% diet DM) of GF. However, when GF was fed at 7.2% of diet DM in corn-silage and haylage-based diet reported no changes in ALA concentration in milk (Petit and Cortes, 2010; Hafla et al., 2018). Overall, feeding EF had consistent effect on milk ALA concentration which increased in most of the studies. The increased ALA concentration may be due to the enhanced intake of ALA and by the fact that extrusion process of flaxseed may have given some level of protection which prevents complete biohydrogenation of ALA.

Whereas the *trans*-11 18:1 and *cis*-9, *trans*-11 18:2 CLA concentration increased ($P < 0.01$) with feeding LIN diet in the current experiment, the concentration of *cis*-9, *cis*-12 18:2 decreased ($P < 0.001$). Similar results were observed when dairy cows fed at incremental levels (0, 2.5, and 5% diet DM) LNPR (Swanepoel and Robinson 2019a). Feeding 21.2% (diet DM) EF product (70% EF and 30% wheat) also increased the concentrations of *trans*-11 18:1 by 84% and *cis*-9, *trans*-11 18:2 CLA by 65% whereas *cis*-9, *cis*-12 18:2 decreased by 5% compared to control diet (Chilliard et al., 2009). Similarly, Ferlay et al. (2013) reported a linear increase in *cis*-9, *trans*-11 18:2 CLA with feeding increasing amounts (0, 5, 10 and 15% diet DM) of EF with either hay or corn silage-based diet. However, when 10% (diet DM) EF was fed either with corn or barley-based diet *trans*-11 18:1 and *cis*-9, *trans*-11 18:2 CLA increased, but no changes in *cis*-9, *cis*-12 18:2 concentration. The decreased concentration of *cis*-9, *cis*-12 18:2 in the

present study could be due to the extensive biohydrogenation, that converts to *cis*-9, *trans*-11 18:2 CLA and *trans*-11 18:1 intermediate as reported by others (Harfoot and Hazlewood, 1988; Jenkins et al., 2007).

The concentration of *trans*-10 18:1 tended to be greater in LIN diet, while no effects was observed for *cis*-9 18:1. A previous study with feeding incremental amount (0, 2.5, and 5% of diet DM) LNPR to the early lactation dairy cows linearly increased the concentration of *cis*-9 18:1 whereas *trans*-10 18:1 quadratically tended to be greater (Swanepoel and Robinson, 2019a). The increased concentration of *trans*-10 18:1 may be due to the extensive biohydrogenation of *cis*-9, *cis*-12 18:2 as explained earlier because *trans*-10 18:1 is the biohydrogenation intermediates of *cis*-9, *cis*-12 18:2. Again, *trans*-10 18:1 can also be formed through alternative biohydrogenation pathway of ALA, although the contribution of ALA to *trans*-10 18:1 is not significant (Baldin et al., 2022).

Intake and Apparent-Total Tract Digestibility of Nutrients

Intake and apparent-total tract digestibility of nutrients are presented in Table 12. Total intake of OM (21.2 vs. 20.3 kg/d), CP (3.82 vs. 3.53 kg/d), NDF (7.55 vs. 6.83), and ADF (5.21 vs. 4.80 kg/d) were greater in CTRL than LIN cows. This increased intake of nutrients in CTRL cows was due to elevated herbage intake (7.39 vs 5.95 kg/d) compared with LIN cows. However, greater ($P \leq 0.02$) apparent total-tract digestibility of nutrients was observed for DM (70.5 vs 69.5%), OM (71.5 vs 70.4%), and CP (65.7 vs 64.8%) with feeding LIN than CTRL. No differences were observed for NDF (mean = 54.3%) and ADF (mean = 53.3%) digestibility between diets. These results are partially supported by Swanepoel and Robinson (2019a) who reported a tendency for OM and NDF digestibility to be higher with feeding incremental amounts (0, 2.5, and 5% diet DM) LNPR. The digestibility of OM, and NDF was also greater

when EF was fed with grass and corn silage-based diet to dairy cows (Gonthier et al., 2004). Feeding EF with either corn or barley increased CP digestibility (Neveu et al., 2014). In contrast, Judy et al. (2019) reported no changes in the digestibility of nutrients when feeding 10.5% (diet DM) of LNPR. Similarly, feeding increasing amounts (0, 5, 10, and 15% of diet DM) EF with hay-based diet did not have any effect on DM, OM, and NDF digestibility but when fed with corn-silage based diet, NDF digestibility decreased linearly (Martin et al., 2016). However, when Martin et al. (2008) fed 21.2% EF product (70% EF and 30% wheat) of diet DM, digestibility of DM and OM was reduced by 5% and NDF digestibility by 25%. This adverse impact of flaxseed supplementation on the digestibility of a diet is more prominent when corn-silage is used as opposed to a diet based on hay as summarized by Martin et al. (2008). The decreased fiber digestibility appears to be more pronounced when EF was used >10% of diet DM, suggesting detrimental effects of PUFA on ruminal microbes (Beauchemin et al., 2007). However, in the current study, fiber digestibility was not affected by the LNPR supplementation, implying that LNPR did not have any negative impact on ruminal fermentation possibly due to the dry extrusion process that was applied during the preparation of this product.

Ruminal Fermentation Profile

Effects of treatments on ruminal fermentation profile are presented in Table 13. Although isobutyrate increased (0.52 vs 0.42 mol/100mol) in LIN versus CTRL at week 7 (treatment × week interaction (P = 0.001), no treatment effects were observed for total VFA concentration (mean = 89.8 mM), and molar proportions of acetate (mean = 71.0%), propionate (mean = 15.3%), butyrate (mean = 11.7%), and the acetate-to-propionate ratio (mean = 4.69). In agreement with our results, the total VFA concentration and molar proportion of individual VFA were not different when cows were fed 11.4% (of diet DM) EF compared to CTRL (Moats et al.,

2018). Also, feeding 10% GF to the grazing cows had no effect on total VFA and molar proportion of other VFA, except butyrate that tended to decrease (Isenberg et al., 2019). Neveu et al. (2014) compared 2 different grain sources (corn vs. barley) with or without EF and reported a decrease in molar proportions of propionate and an increase in butyrate whereas total VFA concentration was greater when EF was fed with corn-based diet as opposed to barley. Contrarily, the molar proportion of propionate increased, and acetate decreased with feeding 12.6% (of diet DM) raw flaxseed, micronized flaxseed, and EF in grass and corn silage-based diet compared to a control (Gonthier et al., 2004). The modest effect observed in the current study may be due to the lower inclusion of LNPR (6% of dietary DM). When incremental levels (0, 5, 10, 15% diet DM) of EF was fed to the dairy cows with hay-based diet, propionate, butyrate, and the acetate-to-propionate ratio decreased linearly, however, total VFA concentration did not change across the diet which is in line with the current study. In their second experiment, when fed the same level of EF with corn-silage based diets, molar proportion of propionate increased linearly or tended to increase quadratically whereas a linear decrease of acetate, butyrate and acetate-to-propionate ratio was observed (Martin et al., 2016). The literature presents inconsistent results regarding the effects of feeding EF on ruminal fermentation parameters. Martin et al. (2016) proposed that the decreased VFA concentration may be associated with the lower fiber digestibility of the diet and the overall changes in VFA concentration was possibly due to the nature of the dietary forages.

Urinary Excretion of Nitrogenous Metabolites

Nitrogen intake and excretion of nitrogenous metabolites are presented in Table 14. Total N intake was greater ($P = 0.05$) in CTRL compared to LIN which was again due to the increased herbage intake in CTRL versus LIN. No treatment differences were observed for the urinary

excretion of N (mean=175 g/d), creatinine (mean = 101 mmol/d), allantoin (mean = 394 mmol/d), uric acid (mean = 64.0 mmol/d), and total PD (mean = 458), and the urinary PD-to-creatinine ratio (mean = 4.60). There is limited data on the effect of EF on urinary excretion of PD in dairy cows, but PD was reported with the studies fed GF. In line with our results, Almeida et al. (2023) also reported no effects on N excretion and urinary purine derivative excretion when fed incremental amounts (0, 5, 10, 15% diet DM) of GF to the dairy cows. Isenberg et al. (2019) fed 10% (diet DM) GF to the grazing dairy cows and observed greater total urinary N excretion, and a tendency to increased urinary creatinine and PD-to-creatinine ratio. Soder et al. (2012) replaced orchardgrass with incremental amounts of GF in continuous culture fermenter and reported no change in microbial flow of N which agrees with the urinary PD excretion data observed herein. Also, urinary N excretion did not appear to have a direct correlation with either PUN or MUN in the current study. Whereas MUN decreased ($P < 0.01$) with feeding LIN versus CTRL, no effects were observed ($P > 0.60$) for PUN or urinary N excretion between the diets. Furthermore, it appears that the urinary N excretion was not influenced by the N intake because treatments had no difference in urinary N excretion when expressed as % of N intake. Yet, the purine derivatives reported herein should be interpreted cautiously because limitations are associated with the accurate estimation of herbage intake as discussed earlier.

Gaseous Emissions

Gaseous emissions are presented in Table 15. No treatment effects were observed for enteric CH_4 production (mean =350 g/d CH_4 yield (mean = 15.2 g/kg of DMI), and CH_4 intensity (mean = 11.1 g/ kg of ECM). Similarly, there were no effects of treatments on CO_2 production (mean = 10.9 kg/d) and O_2 consumption (mean = 8.0 kg/d). In agreement with the current study, Judy et al. (2019) reported no effect on CH_4 production, yield, or intensity when Jersey cows

were fed a CTRL diet and a diet with 10.5% (diet DM) of LNPR. Feeding 5% (diet DM) EF with either corn silage or grass silage diet did not have any effect on CH_4 production or CH_4 yield (Livingstone et al., 2015). Contrarily, when dairy cows were fed 21.2 % (diet DM) EF product (70% EF and 30% wheat), CH_4 production, and CH_4 intensity reduced by 38 and 23%, respectively, compared to a CTRL diet (Martin et al., 2008). Similarly, a linear decrease in daily CH_4 production, CH_4 yield, and intensity was reported with feeding incremental levels (0, 5, 10, 15% diet DM) of EF in hay-based diet (Martin et al., 2016). Also, feeding the same level of EF in a corn-silage based diet tended to decrease daily CH_4 production linearly in addition to a linear reduction of CH_4 yield and intensity (Martin et al., 2016). Compared to EF, studies conducted on GF also decreased CH_4 emissions in dairy cows. Cows fed incremental (0, 5, 10, 15% diet DM) amounts of flaxseed tended to decrease daily CH_4 production (Almeida et al., 2023) where the DMI was also decreased. A linear decrease in CH_4 emissions was observed in our previous study when incremental amounts of GF replaced orchardgrass in a continuous culture (Soder et al. 2012). Collectively, it appears that the reduction in CH_4 emissions was dose-dependent with feeding either EF or GF suggesting that the higher inclusion of flaxseed could potentially lead to a reduction in CH_4 emissions; however, this comes at the expense of lower DMI, milk yield and components and the digestibility of nutrients in dairy cows (Martin et al., 2008; Beauchemin et al., 2009). In the current study, greater digestibility of DM, OM, and CP was observed with feeding LIN. Therefore, the lack of response of feeding LIN on daily CH_4 emission herein may be explained by the lower ruminal degradability and slow release of oil in the rumen due to the dry extrusion process of LNPR, suggesting that the inclusion may not have been great enough to elicit large effect on gaseous emissions as also suggested by Judy et al. (2019).

Energy Partitioning

Energy intake, utilization, and efficiency are presented in Table 16. The GE intake was greater ($P = 0.04$) in CTRL cows (100 vs 96.0 Mcal/d) compared to LIN. Diets had no effects ($P \geq 0.5$) on the intake of DE (mean = 67.5 Mcal/d), ME (mean = 59.8 Mcal/d), and NE_L (mean = 39.5 Mcal/d). There was no diet × week interaction for any of these energy intake variables. Total DMI tended ($P = 0.06$) to be greater (+1.0 kg/d) in CTRL compared to LIN, which may explain the difference in GE intake between diets. The DE intake was observed to be 2.26 and 2.64 Mcal/kg of DM (data not shown) in CTRL and LIN cows, respectively which is consistent with Judy et al. (2019) who reported DE intake 2.73 and 2.80 Mcal/kg of DM, respectively with feeding a control diet and a diet with 10.5% (diet DM) LNPR.

Excretion of fecal energy was lower ($P < 0.01$) in LIN (29.2 vs. 31.4 Mcal/d) compared to CTRL cows which was possibly due to the greater ($P \leq 0.02$) digestibility of DM, OM, and CP with feeding LIN. However, urinary (mean = 2.35 Mcal/d) and CH_4 (mean = 4.63 Mcal/d) energy did not differ ($P \geq 0.73$) between diets. Total urinary N excretion (mean = 175 g/d) did not differ between CTRL and LIN cows, thus supporting the urinary energy excretion result. The CH_4 energy output was observed to be similar (4.67 vs 4.58 Mcal/d) between CTRL and LIN cows which was greater than what Judy et al. (2019) reported (mean = 3.32 mcal/d) when fed 10.5% LNPR of diet DM.

No treatment effects were observed for heat production (**HP**) (mean = 27.9 Mcal/d), milk energy (mean = 22.4 Mcal/d), or tissue energy (mean =9.50 Mcal/d). The HP was calculated using the equation from Brouwer (1965) where consumption of O_2, CO_2, and CH_4 production were used with the total amount of urinary N excretion. Neither CO_2 (g/d) nor CH_4 production were affected by the diets, suggesting no changes in HP. Likewise, no differences were observed

in milk yield and components, BW (mean =475 kg) or BW change, thus indicating no effect on milk or tissue energy between the cows. All other energy efficiency variables did not differ ($P \geq$ 0.71) between treatments.

Conclusion

Except MUN which was decreased, feeding 6% LIN did not affect milk yield and the yield of milk components, thus rejected our first hypothesis that LIN would increase milk yield and yield of milk components. The cows fed LIN diet had greater concentration *cis*-9, *trans*-11 18:2 CLA and Σn-3 FA especially *cis*-9, *cis*-12, *cis*-15 18:3 (ALA) beneficial for human health, whereas it reduced the proportion of ΣSFA which was consistent with our second hypothesis. No treatment effects were observed for CH_4 production, CH_4 yield (g/kg of DMI) or CH_4 intensity (g/kg of ECM) which did not support our third hypothesis. Lastly, our hypothesis was that increasing energy density of the diet by LNPR supplementation would increase DE, ME, and NE_L. Except GE intake and excretion of fecal energy, which was lower in LIN cows, no energy related variables (i.e., intake of DE, ME, NE_L, ME/DE%, milk energy/ME%, or tissue energy/ME%) were different in the current study thereby rejecting the last hypothesis. However, a greater apparent total-tract digestibility of DM, OM, and CP was observed but it did not translate more milk yield or increase energy utilization efficiency of cows. Further research is needed with the higher inclusion (i.e., > 6%) of LNPR to evaluate production performance, enteric CH_4 emissions, and energy utilization in grazing dairy cows.

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Table 1. Ingredient composition of LinPRO-R (Adapted from Moats et al., 2015).

Ingredients	% of DM
Whole Flaxseed	54.7
Ground field peas	37.8
Dehydrated alfalfa	6.97
Vitamin E[1]	0.10
Mold inhibitor[2]	0.30
Ethoxyquin[3]	0.05

[1]Vitamin E: Microvit® (min 500 IU/g; Adisseo, Alpharella, CA)
[2]Mold inhibitor: No mold85 (85% propionic acid; Agri-Marketing Corp; Mont. St. Hilaire, QB)
[3]Ethoxyquin: Santoquin® (min. 91% ethoxyquin; Novus International, Inc.; St. Charles, MO.)

Table 2. Ingredient composition of the experimental diets

	Diet[1]	
Ingredients (% of DM)	CTRL	LIN
Pasture, mixed mostly grass	37.00	37.40
Baleage, mixed mostly legume	23.60	23.60
LinPRO-R[2]	0.00	6.00
Ground corn	25.93	22.73
Extruded soybean	3.02	1.48
Ground barley	5.92	5.23
Roasted soybean	2.38	1.50
NaCl	0.50	0.49
Magox[3]	0.21	0.19
Dikal[4]	0.13	0.12
Limestone[5]	0.16	0.14
Magnesium Sulfate	0.19	0.19
Sodium bicarbonate	0.63	0.61
Yeast	0.07	0.06
Morrison dairy premix	0.26	0.25
CA sulfate granular	-	0.01

[1]Diet supplemented at 0 (CTRL) or 6% (LIN) LinPRO-R of diet DM.
[2]LinPRO-R (O&T Farms, Saskatoon, SK, Canada)
[3]Magox contained 54% Mg.
[4]Dikal contained 19% Ca and 21% P
[5]Limestone contained 35.5% Ca.

Table 3. Ingredient composition of concentrate mash and pellets fed to the grazing dairy cows

Ingredient (% of DM)	CTRL Concentrate mash[1]	LIN Concentrate mash[2]	Chromium pellet[3]	GreenFeed pellet[4]
Alfalfa meal	--	--	--	--
Ground field peas	--	--	--	24.0
Whole flaxseed	--	--	--	--
Dehydrated alfalfa	--	--	--	--
Vitamin E	--	--	--	--
Mold inhibitor	--	--	--	--
Ethoxyquin	--	--	--	--
Ground corn	65.8	68.9	47.8	38.0
Extruded soybean	7.67	4.49	--	--
Ground barley	15.0	15.9	13.0	8.00
Roasted soybean	6.00	4.50	5.00	--
Soybean meal	--	--	6.50	5.50
Soy hulls	--	--	19.5	--
Wheat midds	--	--	--	16.0
Molasses	--	--	3.00	2.00
Fine lime	--	--	--	2.42
Dynamite	--	--	--	0.24
Geobond	--	--	0.5	0.4
Chromium oxide[5]	--	--	1.12	--
NaCl	1.27	1.47	1.25	1.12
Magox[6]	0.53	0.58	0.48	0.49
Dikal[7]	0.33	0.35	--	--
Limestone[8]	0.40	0.42	1.25	--
Magnesium Sulfate	0.49	0.57	--	--
Sodium bicarbonate	1.60	1.85	--	1.25
Yeast	0.17	0.19	--	--
Morrison dairy premix	0.65	0.75	0.60	0.59
CA sulfate granular	-	0.02	--	--

[1]CTRL concentrate mash (10% CP) was included in pTMR for the CTRL diet.
[2]LIN concentrate mash (12% CP) was included in pTMR for the LIN diet.
[3]Chromium pellet (Morrison's Custom Feeds, Barnet, VT)
[4]GreenFeed pellet (Morrison's Custom Feeds, Barnet, VT)
[5]Chromium oxide (City Chemicals LLC, West Heaven, CT) was incorporated with pellet.
[6]Magox contained 54% Mg.
[7]Dikal contained 19% Ca and 21% P
[8]Limestone contained 35.5% Ca.

Table 4. Nutrient composition of (% of DM, unless otherwise noted) concentrate mash, LinPRO-R (LNPR), Cr_2O_3 pellet, GreenFeed (GFeed) pellet, and baleage (mean ± SD) used in the experiment.

Item	CTRL Concentrate mash[1]	LIN Concentrate mash[2]	CTRL pTMR	LIN pTMR	LinPRO-R[3]	Cr_2O_3-pellet[4]	GFeed pellet[5]	Baleage[6]
DM, % of fresh matter	92.5 ± 0.55	92.7 ± 0.17	60.0 ± 1.95	61.4 ± 1.69	95.1 ± 0.40	94.5 ± 0.15	93.4 ± 0.20	67.7 ± 3.09
CP	14.2 ± 0.59	11.7 ± 0.42	15.8 ± 0.58	15.2 ± 0.15	21.0 ± 0.78	14.8 ± 0.32	15.3 ± 0.51	18.5 ± 0.65
Soluble protein, % of CP	16.6 ± 3.79	16.0 ± 0.00	25.9 ± 1.82	26.9 ± 1.09	30.0 ± 1.73	12.6 ± 2.52	30.3 ± 3.21	41.3 ± 2.52
ADICP[7]	0.23 ± 0.06	0.20 ± 0.00	0.60 ± 0.04	0.58 ± 0.04	0.40 ± 0.10	0.30 ± 0.00	0.20 ± 0.00	1.17 ± 0.12
NDICP[8]	1.17 ± 0.21	1.03 ± 0.21	2.58 ± 0.28	2.47 ± 0.15	1.47 ± 0.15	1.93 ± 0.31	1.13 ± 0.21	4.73 ± 0.55
ADF	4.43 ± 0.15	3.50 ± 0.66	18.2 ± 0.37	18.1 ± 0.26	8.33 ± 0.35	6.23 ± 0.25	7.30 ± 1.83	41.2 ± 0.82
aNDFom[9]	8.90 ± 0.90	7.53 ± 0.76	24.1 ± 0.56	23.8 ± 0.56	12.5 ± 1.08	10.9 ± 3.35	12.0 ± 0.89	49.6 ± 1.16
NFC[10]	65.4 ± 1.56	65.3 ± 7.65	47.6 ± 1.27	45.2 ± 3.62	42.4 ± 1.44	59.3 ± 0.46	59.3 ± 1.53	17.8 ± 1.33
Starch	52.9 ± 2.85	55.7 ± 2.79	34.5 ± 2.61	33.1 ± 2.18	25.0 ± 2.03	46.8 ± 2.00	46.0 ± 1.23	3.60 ± 2.65
ESC[11]	3.57 ± 0.51	4.03 ± 0.76	3.4 ± 0.44	3.77 ± 0.46	5.07 ± 0.06	3.97 ± 0.61	4.83 ± 0.85	3.07 ± 0.38
Ether extract	3.66 ± 0.26	3.51 ± 0.09	3.4 ± 0.16	5.06 ± 0.05	22.2 ± 0.02	3.44 ± 0.01	2.16 ± 0.05	2.87 ± 0.00
Ash	7.70 ± 1.67	8.68 ± 0.86	8.9 ± 0.67	9.06 ± 0.30	3.89 ± 0.19	9.73 ± 0.78	10.9 ± 1.34	10.9 ± 1.00
Total fatty acids (FA)	2.34 ± 0.02	2.02 ± 0.26	1.80 ± 0.03	2.66 ± 0.19	13.57 ± 0.48	1.71	1.22	0.79 ± 0.10
FA, g/100 g of total FA								
16:0	20.7 ± 0.85	21.8 ± 1.50	24.6 ± 0.58	23.9 ± 1.20	7.79 ± 0.13	27.3	24.1	30.9 ± 2.08
18:0	4.68 ± 0.12	4.48 ± 0.23	4.80 ± 0.46	4.75 ± 0.47	5.39 ± 0.17	5.13	5.22	4.97 ± 1.26
cis-9 18:1	29.2 ± 0.86	29.2 ± 0.68	20.9 ± 0.36	20.0 ± 0.44	20.3 ± 0.21	33.8	32.9	7.04 ± 1.94
cis-9, cis-12 18:2	0.55 ± 0.02	37.5 ± 2.25	31.9 ± 0.86	29.5 ± 1.50	18.1 ± 0.54	25.3	28.8	21.3 ± 2.18
cis-9, *cis*-12, *cis*-15 18:3	2.05 ± 0.07	1.95 ±0.19	12.1 ±0.94	16.41 ± 0.94	46.2 ± 0.72	2.25	2.29	29.0 ± 2.55

[1]CTRL Concentrate mash was included in pTMR for the CTRL diet: Consisted of (DM basis) 65.81% corn meal, 7.67% extruded soybeans, 15.02% barley grains, 6.00% roasted soybeans, 1.27% plain salt, 0.53% magox (54% mg), 0.33% Dikal-21, 0.40% limestone, 0.49% magnesium sulfate, 1.60% sodium carbonate, 0.17% XP yeast, and 0.65% Morrison dairy premix.

[2]LIN Concentrate mash was included in pTMR for the LIN diet: Consisted of (DM basis) 68.89% corn meal, 4.49% extruded soybeans, 15.85% barley grains, 4.50% roasted soybeans, 1.47% plain salt, 0.58% magox (54% mg), 0.35% Dikal-21, 0.42% limestone, 0.57% magnesium sulfate, 1.85% sodium carbonate, 0.19% XP yeast, 0.75% Morrison dairy premix, and 0.02% CA sulfate granular.

[3]LinPRO-R was fed at 6% of diet DM in LIN diet and separately added with all other ingredients in the mixer.

[4]Cr_2O_3 (City Chemicals LLC, West Heaven, CT) was incorporated with the pellet (Morrison's Custom Feeds, Barnet, VT)

[5]GFeed pellet (Morrison's Custom Feeds, Barnet, VT) was used as bait to attract cows in a portable automated head chamber unit (GreenFeed System, C-Lock Inc., Rapid City, SD) to measure gaseous emissions (Data is shown in Table. 15).

[6]Mixed mostly legume baleage: Consisted of 70% Alfalfa, 14% orchardgrass, 8% timothy, 7% tall fescue, and 1% red clover was harvested and preserved in a plastic wrapped bale.

[7]ADICP = acid detergent insoluble CP

[8]NDICP = neutral detergent insoluble CP

[9]aNDFom = ash free neutral detergent fiber organic matter

[10]NFC = 100 – (CP + NDF + ether extract + ash)

[11]ESC = ethanol-soluble carbohydrate

Table 5. Fatty acid composition of LinPRO-R (LNPR) fed to the grazing dairy cows.

Fatty acid	LinPRO-R[1]
	…..g/100 g of total FA….
Total FA (%)	13.50 ± 0.48
C14:0	0.07 ± 0.01
16:0	7.79 ± 0.13
cis-9 16:1	0.10 ± 0.02
17:0	0.08 ± 0.02
18:0	5.39 ± 0.17
trans- 18:1	0.39 ± 0.14
cis-9 18:1	20.3 ± 0.21
cis-11 18:1	1.14 ± 0.04
cis-9, *cis*-12 18:2	18.1 ± 0.54
cis-6, *cis*-9, *cis*-12 18:3	0.22 ± 0.01
cis-9, *cis*-12, *cis*-15 18:3 (ALA)[2]	46.2 ± 0.72

[1]LinPRO-R is an extruded flaxseed-based product (O&T Farms, SK. Canada)
[2]ALA = α-linolenic acid

Table 6. NASEM (2021) evaluation of the experimental diets

	Diet[1]	
Item[2]	CTRL	LIN
NDF, % DM	32.9	32.8
Forage NDF, % DM	29.4	29.5
ADF, % DM	23.2	23.2
ME[3], Mcal/kg DM	2.57	2.68
DCAD[4], mEQ/kg	333	334
NE_L[5], Mcal/kg DM	1.70	1.77
NE_L required, Mcal/d	34.8	34.8
NE_L supplied, Mcal/d	32.3	33.7
NE_L balance, Mcal/d	-2.53	-1.11
MP[6] required, g/d	1680	1680
MP supplied, g/d	1727	1706
MP balance, g/d	47	26
DM intake-actual, kg/d	21.9	21.8
DM intake-predicted, kg/d	19.0	19.0
NE_L allowable milk, kg/d	20.5	22.0
MP allowable milk, kg/d	24.6	24.1
Actual milk, kg/d	27.6	27.1
CP, % DM	16.4	16.0
RDP[7], % DM	11.6	11.3
RUP[8], % DM	4.8	4.7

[1]Diet supplemented with LinPRO-R at 0 (CTRL) or 6% (LIN) of diet DM.
[2]Values predicted here using the NASEM (2021) dairy diet evaluation software.
[3]ME = Metabolizable energy
[4]DCAD = Dietary cation anion difference
[5]NE_L = Net energy of lactation
[6]MP = Metabolizable protein
[7]RDP = Rumen degradable protein
[8]RUP = Rumen undegradable protein

Table 7. Herbage biomass, allowance, chemical composition, and pasture botanical and nutrient composition throughout the grazing season

	Experimental Week[1]		
Item	Wk 4	Wk 7	Wk 10
Average air temperature, °C	24.1 (19.1-30.8)	20.9 (14.8-27.3)	13.7 (14.3-26.7)
Average relative humidity, %	75.2 (51.0-91.2)	73.1 (49.3-90.3)	78.6 (60.9-90.2)
Pre-grazing herbage mass, kg of DM/ha	2,563	2,221	1,362
Post-grazing herbage mass, kg of DM/ha	1,593	1,768	1,011
Pre-grazing herbage height, cm	34.0	25.4	25.3
Post-grazing herbage height, cm	23.5	14.9	13.4
Daily pasture area, m²/cow	62.9	62.6	108.5
Daily herbage allowance,[2] kg of DM/cow	16.1	14.0	14.8
Pasture botanical composition,[3] % of DM			
Grasses	51.6	43.1	54.2
Legumes	15.1	17.8	10.8
Weeds	11.0	18.0	15.9
Dead materials	22.1	20.9	18.9
Nutritional composition, % of DM			
DM, % of fresh matter	38.8	35.1	20.3
CP	12.0	18.4	22.0
Soluble CP protein, % of CP	28.5	24.5	27.5
aNDFom	55.5	55.7	47.7
ADF	35.2	35.3	31.6
Lignin	7.15	7.10	10.3
Ether extract	5.31	6.01	5.55
Ash	7.49	9.13	8.95
ADICP[4]	0.95	1.75	1.80
NDICP[5]	2.85	7.05	5.60
Starch	1.65	0.70	1.25
ESC[6]	5.95	4.45	9.45
NE_L[7] (Mcal/kg of DM)	1.29	1.31	1.43

[1]Wk 4 (August 5 to August 11); Wk 7 (August 26 to September 1); Wk 10 (September 16 to September 22)
[2]Daily herbage allowance = [pregrazing herbage mass (kg of DM/ha) × pasture area (m^2/cow per day)]/10,000.
[3]Predominant herbage species found in the paddocks were orchardgrass (*Dactylis glomerata* L.), white clover (*Trifolium repens* L.), red clover (*Trifolium pratense* L.), perennial ryegrass (*Lolium perenne* L.), and Timothy (*Phleum pratense* L.).
[4]ADICP = acid detergent insoluble CP.
[5]NDICP = neutral detergent insoluble CP.
[6]ESC = ethanol-soluble carbohydrates
[7]NE_L = Net energy of lactation

Table 8. Mineral composition, and fatty acid profile of herbage samples throughout the experiment

	Experimental Week[1]		
Item	Wk 4	Wk 7	Wk 10
Ca	0.75	0.66	0.51
P	0.33	0.30	0.37
Mg	0.26	0.30	0.25
K	1.97	3.21	3.21
Na	0.02	0.03	0.03
S	0.24	0.26	0.28
Cl	0.63	0.90	1.29
Fe, mg/kg of DM	74.0	129	152
Zn, mg/kg of DM	24.5	34.5	35.5
Cu, mg/kg of DM	5.0	8.50	9.50
Mn, mg/kg of DM	37.5	47.0	52.0
Mo, mg/kg of DM	4.0	6.5	2.8
DCAD[2], mEq/100 g of DM	18.5	42.5	30.0
FA, g/100 g of total FA			
16:0	21.6	17.7	15.09
18:0	3.69	2.26	1.85
cis-9 18:1	5.23	3.79	2.73
cis-9, cis-12 18:2	21.5	21.2	17.0
cis-9, *cis*-12, *cis*-15 18:3 (ALA)[3]	39.0	49.1	58.8

[1]Wk 4 (August 5 to August 11); Wk 7 (August 26 to September 1); Wk 10 (September 16 to September 22)
[2]DCAD = Dietary Cation anion difference
[3]ALA = α-linolenic acid

Table 9. Least square means for DMI, milk yield, milk composition, BW, and BCS in Jersey cows grazing cool-season perennial herbage supplemented with a partial TMR (pTMR) plus 0% (control diet = CTRL) or 6% LinPRO-R (LinPRO diet = LIN) of diet DM

	Treatment			*P-value*[1]		
Item	CTRL	LIN	SEM	TRT	Week[2]	T × W
Estimated herbage DMI[3], kg/d	7.39	5.95	0.41	<0.01	0.03	0.01
pTMR DMI, kg/d	14.5	14.9	1.01	0.14	<0.01	0.90
Cr_2O_3 pellet DMI[4], kg/d	0.84	0.84	--	--	--	--
GreenFeed pellet DMI[5], kg/d	0.55	0.56	0.45	0.99	<0.01	0.78
Total supplement DMI[6], kg/d	15.9	16.3	1.01	0.07	<0.01	0.95
Total DMI, kg/d	23.3	22.3	0.51	0.06	<0.01	0.06
Milk yield, kg/d	27.6	27.1	0.91	0.68	<0.01	0.55
4% FCM yield[7], kg/d	30.3	29.5	1.07	0.62	<0.01	0.77
ECM yield[8], kg/d	32.5	31.8	1.11	0.68	<0.01	0.87
Milk yield/DMI, kg/kg	1.21	1.23	0.05	0.53	0.64	0.84
4% FCM/DMI, kg/kg	1.27	1.37	0.04	0.06	0.03	0.22
ECM/DMI, kg/kg	1.37	1.47	0.04	0.05	0.02	0.20
Milk fat, %	4.54	4.60	0.13	0.47	<0.01	0.08
Milk fat, kg/d	1.26	1.27	0.04	0.78	<0.01	0.74
Milk true protein, %	3.49	3.46	0.05	0.67	<0.01	0.56
Milk true protein, kg/d	0.95	0.95	0.03	0.74	<0.01	0.54
Milk lactose, %	4.78	4.80	0.01	0.40	<0.01	0.61
Milk lactose, kg/d	1.31	1.32	0.04	0.76	<0.01	0.72
Milk SNF, %	9.20	9.22	0.04	0.68	<0.01	0.63
Milk SNF, kg/d	2.53	2.54	0.08	0.92	<0.01	0.52
Milk TS, %	13.8	13.7	0.17	0.85	<0.01	0.13
Milk TS, kg/d	3.81	3.77	0.12	0.83	<0.01	0.75
MUN, mg/dL	11.0	8.38	0.39	<0.01	<0.01	0.27
PUN, mg/dL	21.0	21.7	0.87	0.62	0.01	0.28
Milk SCC[9], × 1,000 cells	87.0	75.7	1.19	0.57	0.04	0.69
BW, kg	473	478	4.39	0.30	<0.01	0.49
BW change, kg/d	-0.18	-0.21	0.08	0.78	<0.01	0.36
BCS	2.51	2.55	0.02	0.21	<0.01	0.79
BCS change	-0.0001	-0.0004	0.001	0.81	<0.01	0.42

[1]Significant difference between diets was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.
[2]Wk 4 (August 5 to August 11); Wk 7 (August 26 to September 1): Wk 10 (September 16 to September 22)
[3]Estimated herbage DMI (kg/d) = {fecal DM output (kg/d) − [pTMR DMI (kg/d) × 1 − pTMR 48-h IVDMD]}/ (1 − herbage 48-h IVDMD) (Bargo et al., 2002a).
[4]A fixed amount (0.84 kg/d) of Cr_2O_3 pellet was fed to each experimental cow for 10 d; consumption of the pellet was confirmed visually.
[5]GreenFeed pellet (Morrison's Custom Feeds, Barnet, VT) was used as bait to attract cows in a portable automated head chamber unit (GreenFeed System, C-Lock Inc., Rapid City SD) to measure gaseous emissions (Data is shown in Table. 13).
[6]Total supplement DMI = pTMR DMI (kg/d) + Cr_2O_3 containing pellet DMI (kg/d) + Alfalfa pellet DMI (kg/d).
[7]4% FCM yield = [0.40 × milk yield (kg/d)] + [15 × milk fat yield (kg/d)] (Gaines and Davidson, 1923).
[8]ECM yield = [0.327 × milk yield (kg/d)] + [12.95 × milk fat yield (kg/d)] + [7.2 × milk true protein yield (kg/d)] (Orth, 1999]
[9] Log-transformed data was used to perform statistical analysis.

Table 10. Least square means for milk proportion of saturated fatty acids (SFA) in Jersey cows grazing cool-season perennial herbage supplemented with a partial TMR (pTMR) plus 0% (control diet = CTRL) or 6% LinPRO-R (LinPRO diet = LIN) of diet DM

	Wk 4		Wk 7		Wk 10			*P-value*[1]		
FA, g/100 g of total milk	CTRL	LIN	CTRL	LIN	CTRL	LIN	SEM	TRT	Week[2]	T × W
4:0	4.97	4.91	4.95	5.06	4.80	4.78	0.09	0.93	0.01	0.48
6:0	2.83	2.65	2.86	2.74	2.74	2.64	0.04	<0.01	0.05	0.65
8:0	1.64	1.50	1.65	1.54	1.57	1.49	0.03	<0.01	0.17	0.64
9:0	0.03	0.02	0.03	0.03	0.03	0.03	0.002	0.29	0.02	0.71
10:0	3.75	3.40	3.75	3.32	3.56	3.41	0.10	<0.01	0.42	0.14
11:0	0.05	0.05	0.06	0.05	0.07	0.06	0.004	0.05	0.02	0.80
12:0	4.22	4.03	4.25	3.76	4.03	3.94	0.13	0.07	0.79	0.08
13:0	0.09	0.07	0.09	0.08	0.10	0.09	0.004	0.01	0.05	0.66
iso 13:0	0.03[a]	0.02[b]	0.030	0.02	0.03[a]	0.04[b]	0.001	0.79	<0.01	<0.01
anteiso 13:0	0.08	0.06	0.09	0.07	0.09	0.09	0.003	<0.01	0.15	0.15
14:0	11.6	11.0	11.8[a]	10.8[b]	11.2	11.1	0.20	0.05	0.27	<0.01
iso C14:0	0.09	0.08	0.09	0.07	0.01	0.09	0.003	0.02	<0.01	0.33
15:0	0.97	0.87	0.94	0.83	0.98	0.94	0.02	<0.01	0.08	0.46
iso 15:0	0.22[a]	0.21[b]	0.21[a]	0.12[b]	0.23	0.23	0.003	<0.01	<0.01	0.02
anteiso 15:0	0.42[a]	0.38[b]	0.41[a]	0.36[b]	0.41	0.41	0.009	<0.01	<0.01	0.01
16:0	28.5[a]	26.4[b]	29.2[a]	26.2[b]	26.7	26.1	0.54	0.01	<0.01	<0.01
iso 16:0	0.18	0.20	0.21	0.19	0.18	0.17	0.005	0.47	0.03	0.09
17:0	0.49	0.44	0.48	0.45	0.44	0.44	0.007	0.45	<0.01	0.14
iso 17:0	0.44	0.45	0.43	0.44	0.45	0.48	0.01	0.05	0.05	0.44
anteiso 17:0	0.37	0.37	0.37	0.35	0.30	0.30	0.008	0.48	<0.01	0.26
18:0	11.2	11.9	10.5	11.4	11.2	11.2	0.34	0.32	0.12	0.31
24:0	0.04	0.04	0.04	0.03	0.04	0.03	0.001	0.04	0.22	0.26
ΣSFA[3]	68.9[a]	65.9[b]	69.1[a]	65.0[b]	65.8	64.8	0.54	<0.01	<0.01	<0.01
Unidentified	2.1[a]	3.24[b]	2.11[a]	3.87[b]	2.88[a]	3.93[b]	0.05	<0.01	<0.01	<0.01
ΣOdd chain[4]	1.71	1.69	1.68[a]	1.55[b]	1.66	1.68	0.04	0.08	0.02	0.03
ΣBranched Chain[5]	1.89	1.81	1.86[a]	1.70[b]	1.81	1.82	0.03	0.04	0.09	0.03
Σ<16C[6]	30.4	28.5	30.7	28.4	29.4	28.6	0.54	0.01	0.20	0.06
Σ16C[7]	29.4[a]	27.3[b]	30.2[a]	27.1[b]	27.7	27.1	0.57	0.01	<0.01	<0.01
Σ18C[8]	33.7[a]	16.1[b]	32.7[a]	36.0[b]	36.0	35.6	0.67	0.07	0.012	<0.01

[1]Significant difference between diets was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.
[2]Wk 4 (August 5 to August 11); Wk 7 (August 26 to September 1): Wk 10 (September 16 to September 22).
[3]ΣSFA = 4:0 + 6:0 + 8:0 + 10:0 + 12:0 + 14:0 + 16:0 + 18:0 + 24:0
[4]ΣOdd chain FA = 9:0 + 11:0 + 13:0 + 15:0 + 17:0 + *cis*-10 17:1
[5]ΣBranched chain FA = *iso* 13:0 + *anteiso* 13:0 + *iso* 14:0 + *iso* 15:0 + *anteiso* 15:0 + *iso* 16:0 + *iso* 17:0 + *anteiso* 17:0
[6]Σ<16C = 4:0 + 6:0 + 8:0 + 10:0 + *cis*-9 10:1 (see Table 14) + 12:0 + *cis*-12:1 + 14:0 + *cis*-9 14:1
[7]Σ16C 16:0 + cis-9 16:1 (see Table 14).
[8]Σ18C = 18:0 + all 18C UFA (see Table 11).

Table 11. Least square means for milk proportion of unsaturated fatty acids (UFA) in Jersey cows grazing cool-season perennial herbage supplemented with a partial TMR (pTMR) plus 0% (control diet = CTRL) or 6% LinPRO-R (LinPRO diet = LIN) of diet DM

	Wk 4		Wk 7		Wk 10			*P-value1*[1]		
FA, g/100 g of total milk FA	CTRL	LIN	CTRL	LIN	CTRL	LIN	SEM	TRT	Week[2]	T × W
12:1	0.01	0.08	0.10	0.09	0.11	0.10	0.004	0.02	<0.01	0.19
cis-9 14:1	0.85	0.70	0.95	0.80	0.98	0.93	0.02	<0.01	<0.01	0.06
cis-9 16 1	0.87	0.92	0.94	0.98	0.97	1.06	0.04	0.33	0.01	0.64
cis-10 17:1	0.13	0.15	0.13	0.13	0.12	0.13	0.004	0.05	<0.01	0.61
trans-4 18:1	0.02[a]	0.03[b]	0.02[a]	0.031[b]	0.02	0.02	0.001	<0.01	<0.01	<0.01
trans-5 18:1	0.02	0.03	0.01	0.03	0.01	0.02	0.001	<0.01	<0.01	0.07
trans-6-8 18:1	0.28[a]	0.36[b]	0.25[a]	0.32[b]	0.27	0.28	0.006	<0.01	<0.01	<0.01
trans-9 18:1	0.22[a]	0.28[b]	0.21[a]	0.28[b]	0.23	0.23	0.004	<0.01	<0.01	<0.01
trans-10 18:1	0.34[a]	0.42[b]	0.35	0.40	0.32	0.28	0.011	0.08	<0.01	0.01
trans-11 18:1	1.24	1.57	0.81[a]	1.29[b]	1.80	1.99	0.08	<0.01	<0.01	0.01
trans-11/*trans*-10 18:1	3.07	3.02	2.42[a]	2.88[b]	5.85[a]	7.06[b]	0.18	0.03	<0.01	0.04
trans-12 18:1	0.38[a]	0.50[b]	0.38[a]	0.61[b]	0.39	0.40	0.01	<0.01	<0.01	<0.01
cis-9 18:1	15.1	15.2	15.3	16.0	16.7	16.5	0.43	0.64	<0.01	0.08
cis-11 18:1	0.68[a]	0.81[b]	0.63[a]	0.86[b]	0.64[a]	0.73[b]	0.01	<0.01	<0.01	<0.01
cis-12 18:1	0.35[a]	0.64[b]	0.42[a]	0.92[b]	0.35	0.39	0.01	<0.01	<0.01	<0.01
cis-9, *cis*-12 18:2	2.63[a]	2.29[b]	2.74[a]	2.36[b]	2.37[a]	1.84[b]	0.03	<0.01	<0.01	0.04
cis-9, *trans*-11 18:2	0.46	0.52	0.36	0.50	0.76	0.83	0.02	<0.01	<0.01	0.46
cis-9, *cis*-12, *cis*-15 18:3 (ALA)[3]	0.54[a]	0.88[b]	0.50[a]	0.95[b]	0.71[a]	0.96[b]	0.01	<0.01	<0.01	<0.01
cis-11 20:1	0.01	0.09	0.10	0.08	0.01	0.09	0.09	<0.01	0.87	0.12
20:2, n-6	0.03	0.03	0.03	0.02	0.02	0.01	0.001	<0.01	<0.01	0.33
20:3, n-6	0.12	0.09	0.13	0.09	0.12	0.09	0.003	<0.01	0.02	0.43
20:4, n-6	0.11[a]	0.09[b]	0.13[a]	0.09[b]	0.11[a]	0.08[b]	0.005	<0.01	<0.01	<0.01
20:5, n-3	0.06	0.08	0.05	0.07	0.06	0.07	0.003	<0.01	<0.01	0.76
22:3, n-3	0.02	0.02	0.02[a]	0.01[b]	0.01	0.01	0.001	0.65	<0.01	0.01
22:4, n-3	0.02	0.02	0.03	0.02	0.02	0.02	0.02	<0.01	<0.01	0.18
22:5, n-3	0.09	0.10	0.09	0.10	0.07	0.09	0.003	<0.01	<0.01	0.64
22:6, n-3	0.02	0.02	0.02	0.02	0.05[a]	0.06[b]	0.002	0.26	<0.01	0.01
Σ n-6[4]	3.11	2.70	3.24	2.75	2.80	2.20	0.04	<0.01	<0.01	0.06
Σ n-3[5]	0.76[a]	1.12[b]	0.71[a]	1.19[b]	0.91[a]	1.20[b]	0.01	<0.01	<0.01	<0.01
n-6/n-3 ratio	4.03	2.40	4.46	2.31	3.03[a]	1.90[b]	0.03	<0.01	<0.01	<0.01

[1]Significant difference between diets was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.
[2]Wk 4 (August 5 to August 11); Wk 7 (August 26 to September 1): Wk 10 (September 16 to September 22).
[3]ALA = α-linolenic acid.
[1]Significant difference between diets was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.
[2]Wk 4 (August 5 to August 11); Wk 7 (August 26 to September 1): Wk 10 (September 16 to September 22).
[4]Σn-6 FA = *cis*-9, *cis*-12 18 :2 + *cis*-9, *cis*-12 18 :3 + *cis*-20 2n-6, *cis*-20 3n-6, *cis*-20 4n-6
[5]Σn-3 FA = *cis*-9, cis-12, *cis*-15 18:3 + *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17 20:5 + *cis*-22 3n-3 + *cis*-22 4n-3 + *cis*-22 5n-3 + *cis*-22 6n-3

Table 12. Least square means for intake and apparent total tract digestibility in Jersey cows grazing cool-season perennial herbage supplemented with a partial TMR (pTMR) plus 0% (control diet = CTRL) or 6% LinPRO-R (LinPRO diet = LIN) of diet DM

	Treatment			*P-value*[1]		
Item	CTRL	LIN	SEM	TRT	Week[2]	T × W
Intake, kg/d						
OM	21.2	20.3	0.50	0.05	<0.01	0.11
CP	3.82	3.53	0.05	<0.01	<0.01	0.49
NDF	7.55	6.83	0.24	<0.01	<0.01	0.07
ADF	5.21	4.80	0.16	0.01	0.02	0.49
Total tract digestibility, % of intake						
DM	69.5	70.5	0.18	<0.01	<0.01	0.14
OM	70.4	71.5	0.20	<0.01	0.02	0.12
CP	64.8	65.7	0.37	0.02	<0.01	0.02
NDF	54.3	54.3	0.70	0.99	<0.01	0.37
ADF	53.2	53.5	0.61	0.75	<0.01	0.54

[1]Significant difference between diets was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.
[2]Wk 4 (August 5 to August 11); Wk 7 (August 26 to September 1): Wk 10 (September 16 to September 22).

Table 13. Least square means for ruminal fermentation profile in Jersey cows grazing cool-season perennial herbage supplemented with a partial TMR (pTMR) plus 0% (control diet = CTRL) or 6% LinPRO-R (LinPRO diet = LIN) of diet DM

	Treatment			*P-value*[1]		
Item	CTRL	LIN	SEM	TRT	Week[2]	T × W
Total VFA, mM	88.8	90.9	4.54	0.72	<0.01	0.07
VFA, mol/100 mol						
Acetate (A)	71.2	70.9	0.59	0.72	0.87	0.82
Propionate (P)	15.1	15.5	0.51	0.50	0.20	0.09
Butyrate	11.8	11.5	0.34	0.51	<0.01	0.26
Isobutyrate	0.41	0.43	0.02	0.48	<0.01	<0.01
Valerate	1.18	1.21	0.04	0.70	<0.01	0.53
Isovalerate	0.16	0.18	0.02	0.47	<0.01	0.99
A:P ratio	4.78	4.61	0.21	0.55	0.29	0.20

[1]Significant difference between diets was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.
[2]Wk 4 (August 5 to August 11); Wk 7 (August 26 to September 1): Wk 10 (September 16 to September 22).

Table 14. Least square means for N excretion and urinary purine derivative excretion in Jersey cows grazing cool-season perennial herbage supplemented with a partial TMR (pTMR) plus 0% (control diet = CTRL) or 6% LinPRO-R (LinPRO diet = LIN) of diet DM

	Treatment			*P-value*[1]		
Item	CTRL	LIN	SEM	TRT	Week[2]	T × W
Total N intake, g/d	607	570	15.4	0.05	<0.01	0.49
Urinary creatinine, mM	2.52	2.62	0.08	0.37	0.65	0.89
Urinary creatinine, mmol/d	100	101	0.96	0.54	<0.01	0.19
Urinary uric acid, mmol/d	64.1	63.9	2.30	0.94	<0.01	0.46
Urinary allantoin, mmol/d	392	395	11.0	0.86	0.50	0.79
Urinary total PD[3], mmol/d	456	461	11.7	0.74	0.30	0.67
Urinary PD:creatinine ratio	4.65	4.56	0.15	0.59	0.22	0.48
Total urinary N, g/d	176	174	7.44	0.83	<0.01	0.97
Total urinary N, % of N intake	29.1	30.7	1.64	0.50	0.77	0.74

[1]Significant difference between diets was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.
[2]Wk 4 (August 5 to August 11); Wk 7 (August 26 to September 1): Wk 10 (September 16 to September 22).
[3]PD = purine derivatives (allantoin + uric acid): Calculated with PD:creatinine excretion ratio calculated using an average creatinine excretion of 0.212 mmol/kg of BW (Chizzotti et al., 2008).

Table 15. Least square means for enteric gas emissions in Jersey cows grazing cool-season perennial herbage supplemented with a partial TMR (pTMR) plus 0% (control diet = CTRL) or 6% LinPRO-R (LinPRO diet = LIN) of diet DM

	Treatment			*P-value*[1]		
Item	CTRL	LIN	SEM	TRT	Week[2]	T × W
CH_4 production, g/d	355	348	16.1	0.77	0.31	0.86
CH_4 yield, g/kg of DMI	15.3	15.7	0.69	0.70	<0.01	0.58
CH_4 intensity, g/kg of ECM[3]	11.6	11.0	0.63	0.46	<0.01	0.50
CO_2, kg/d	11.0	10.8	0.28	0.64	0.03	0.40
O_2, kg/d	8.17	7.95	0.20	0.46	0.62	0.69

[1]Significant difference between diets was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.
[2]Wk 4 (August 5 to August 11); Wk 7 (August 26 to September 1): Wk 10 (September 16 to September 22).
[3]ECM yield = [0.327 × milk yield (kg/d)] + [12.95 × milk fat yield (kg/d)] + [7.2 × milk true protein yield (kg/d)] (Orth, 1999]

Table 16. Least square means for energy utilization and efficiency in Jersey cows grazing cool-season perennial herbage supplemented with a partial TMR (pTMR) plus 0% (control diet = CTRL) or 6% LinPRO-R (LinPRO diet = LIN) of diet DM

	Treatment			*P-value*[1]		
Item	CTRL	LIN	SEM	TRT	Week[2]	T × W
Fractions[3], Mcal/d						
GE intake	100	96	2.15	0.04	<0.01	0.06
DE intake	68.7	66.4	1.44	0.14	<0.01	0.10
ME intake	60.6	59.0	1.03	0.45	<0.01	0.09
NE_L intake[4]	40.2	38.9	1.03	0.50	<0.01	0.11
Components, Mcal/d						
Fecal energy[5]	31.4	29.2	0.77	<0.01	<0.01	0.05
Urinary energy[6]	2.39	2.30	1.12	0.83	0.63	0.52
CH_4 energy[7]	4.67	4.58	0.22	0.77	0.31	0.86
Heat production[8]	28.3	27.7	0.71	0.53	0.56	0.87
Milk energy[9]	22.4	22.6	0.89	0.87	<0.01	0.16
Tissue energy[10]	10.1	8.90	0.50	0.87	0.03	0.64
Efficiencies, %						
ME/DE	89.1	89.0	0.48	0.87	<0.01	0.44
Milk energy/ME	36.8	37.5	1.33	0.71	0.01	0.04
Heat production/ME	46.8	47.0	1.03	0.87	0.02	0.13
Tissue energy/ME	19.7	19.0	1.10	0.86	0.13	0.02

[1]Significant difference between diets was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.
[2]Wk 4 (August 5 to August 11); Wk 7 (August 26 to September 1): Wk 10 (September 16 to September 22).
[3]GE = gross energy; digestible energy (DE) intake (Mcal/d) = GE intake (Mcal/d) − fecal energy (Mcal/d); ME intake (Mcal/d) = DE intake (Mcal/d) − urinary energy (Mcal/d) − CH4 energy (Mcal/d) (NRC, 2001).
[4]NE_L intake (Mcal/d) = ME intake (Mcal/d) × 0.66
[56]Gross energy of feces and urine was estimated by parr 6200 (Parr instrument company, Moline, IL) oxygen bomb calorimeter.
[7]Methane energy was calculated by multiplying CH_4 production (L/d) by CH_4 enthalpy (9.45 Kcal/L).
[8]HP (Kcal/d) = 3.866 × O_2 (L/d) + 1.200 × CO_2 (L/d) – 0.518 × CH_4 (L/d) – 1.431 × urinary N excretion (g/d) (Brouwer, 1965)
[9]Milk energy (Mcal/d) = [(0.0929 × milk fat%) + (0.0585 × milk true protein%) + (0.0395 × milk lactose%)] × milk yield (kg/d). [NSEM. 2021].
[10]Tissue energy (Mcal/d) = ME intake (Mcal/d) – heat production (Mcal/d) – milk energy (Mcal/d).

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14-Jul-2022

Brito, Andre Fonseca De
Agriculture, Nutrition, & Food Systems
Keener Dairy Research Building
Durham, NH 03824

IACUC #: 220602
Project: Effects of Flaxseed-based Feed Supplement on Production Performance, Energy Utilization, and Milk Fatty Acid Profile in Jersey Cows Grazing Mixed Grass-legume Pasture
Approval Date: 14-Jul-2022

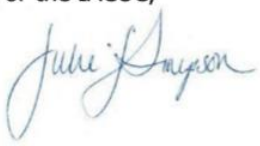
The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category C in Section V of the Application for Review of Vertebrate Animal Use in Research or Instruction - *Animal use activities that involve either no pain or potentially involve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

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

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

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

For the IACUC,



Julie Simpson, Ph.D.
Director

cc: File