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Katie Velez

University of New Hampshire - Main Campus, kll486@wildcats.unh.edu

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Effects of Incremental Dietary Levels of Ground Flaxseed on Milk
Production, Ruminal Metabolism, and Enteric Methane Emissions in
Organic Dairy Cows

Katie Velez
Faculty Mentor: Dr. André F. Brito

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Abstract

Ground Flaxseed (*Linum uitatissimum*) is a lipid supplement that is commonly fed to dairy cows. It is believed that supplemental lipid can change the Fatty Acid (FA) composition in the milk, and decrease methane production. Twenty lactating organic Jersey cows, housed at the UNH Organic Dairy Research Farm (ODRF), were randomly assigned to five replicated 4 × 4 Latin squares to investigate the effects of increasing dietary levels of ground flaxseed (0, 5, 10, or 15% of the diet dry matter) on animal performance (e.g., dry matter intake, milk production, milk composition), ruminal metabolism, and enteric methane emissions. Each period lasted 21 days with 14 days for diet adaptation and seven days for data and samples collection. Cows were fed twice daily (a.m. and p.m.) a total mixed ration containing 65% grass-legume baleage, and one of the following supplemental mixtures:

- 0% ground flaxseed, 27% corn meal, and 8% soybean meal
- 5% ground flaxseed, 24% corn meal, and 6% soybean meal
- 10% ground flaxseed, 21% corn meal, and 4% soybean meal
- 15% ground flaxseed, 17.5% corn meal, and 2.5% soybean meal.

Feeding incremental dietary levels of ground flaxseed resulted in linear decreases of dry matter intake, yields of milk and milk components, ruminal molar proportion of acetate and butyrate, and enteric methane emissions. However, the molar proportion of propionate increased linearly with feeding incremental dietary levels of ground flaxseed. Further research is needed to investigate the long-term effects of ground flaxseed on milk yield and animal health.

Introduction

Organic milk production has been one of the fastest growing segments of organic agriculture in the US in the last decade (McBride and Green, 2009). Forty-four percent of all US certified organic dairies are located in the Northeast, accounting for 25% of the total organic milk produced in the country (McBride and Green, 2009). In New England, organic has proved to be an invaluable option to preserve farm businesses, as 75% of the farmers believe they would be out of business if they had not transitioned to organic.

There have been numerous studies done on supplementing lipids, including flaxseed, to lactating dairy cows. Researchers have been looking at how flaxseed modify the fatty acid (FA) profiles of cow's milk as well as its effect on milk production, cow health, and enteric methane (CH₄) emissions.

Recently, milk FA composition has been used to predict enteric CH₄ output in lactating dairy cows due to the common bio-chemical pathways between CH₄, acetate, and butyrate in the rumen (Beauchemin et al., 2011). Enteric CH₄ produced by ruminants represents an energy loss to the animal and it contributes to the greenhouse gas effect and global warming (Beauchemin et al., 2011). Approximately 2 to 12% of the gross energy intake is converted into CH₄ (Beauchemin et al., 2008). Previous studies have been shown that oilseed-supplemented diets decreased CH₄ production (g/d) by 13% on average (Beauchemin, et al., 2011). Using nonruminally-protected dietary lipids is another nutritional aspect that has been looked at and shown to lower CH₄ emissions (Beauchemin et al., 2008). It is believed that added fats decrease CH₄ emissions because they lower the amount of organic matter that is fermented in the rumen, the activity of the ruminal methanogens, and protozoal numbers (Beauchemin, et al., 2008). In addition, the ability of supplemental fats for suppressing CH₄ could be due to multiple factors such as the amount of the supplement added to the diet, the total fat concentration of the diet, FA profile of the supplement fat source, the form of the fat that is administered, and the diet composition (Beauchemin et al., 2008). In other cases, it is thought that CH₄ emissions are lowered because the added fats lower the dry matter intake (DMI), the

diet digestibility, or both (Beauchemin et al., 2008). Thus, it has been a concern that if the added fats lower the DMI and digestibility that there will be a subsequent decrease in milk production.

Adding lipids to the diet for CH₄ mitigation is very likely since lipid sources are a common additive to increase the energy density of dairy diets (Beauchemin et al., 2008). Also because when fats are high in polyunsaturated FA, it can alter the FA composition in milk as it has been previously studied. The alteration in the milk FA composition can be beneficial to human health by increasing proportions of monounsaturated FA and polyunsaturated FA, and by increasing the concentrations of conjugated linoleic acids (CLA) (Beauchemin et al., 2008). Nonetheless, different fat sources need to be evaluated and the effect on milk yield and composition need to be further investigated and well established before dairy farmers are likely to use supplemented lipids to mitigate CH₄ emissions (Beauchemin, et al., 2008).

Literature Review

Chilliard et al. (2009) investigated the effects of feeding linseed to lactating dairy cows and the effects it had on milk yield and FA composition. They used 8 lactating multiparous Holstein cows that were blocked according to their physiological stage. Each cow was assigned to 4

dietary treatments in a replicated 4 x 4 Latin square design with each experimental period lasting 4 weeks. The four different treatments were the control diet (C), control with whole crude linseed (CLS), control with extruded linseed (ELS) and control with linseed oil (LSO). Each diet was formulated to have similar oil content (Chilliard et al., 2009).

They showed that CLS had no effect on the silage and concentrate intakes or the dry matter intake whereas the ELS and LSO both decreased the dry matter intake when compared to the C. Milk yield was lowest for LSO followed by ELS. CLS and C did not differ from one another. The milk fat content was higher in CLS and lower in both the ELS and the LSO compared to C. Protein and lactose yields on the other hand, did not vary among the different diets, nor did the body weight of the cows. When the FA composition was looked at, the CLS diet had slight differences when compared to C. In the CLS diet, Milk C8:0 to 16:0 were decreased, but odd and branched – chain FA did not change except for a slight decrease in 17:0. There was also a large increase in 18:0 and cis-9 18:1 and a small increase in 20:0 and trans-16 +cis-14 18:1. A small decrease was also seen with cis-9, cis-12 18:2 and cis-9, cis-11 CLA. LSO on the other hand when compared to C decreased all of the FA except 5:0 from 4:0 to 16:0. It increased 18:0, trans-11 16:1, and all trans 18:1 except for trans-11 18:1. It didn't change the

concentration of CLA isomers except for a minor decrease in cis-9, cis-11 CLA. The overall effect of LSO was a decrease in saturated FA and an increase in unsaturated FA. ELS was an intermediate between CLS and LSO when controlled to C with a few differences. ELS diets had an increase in 17:0, trans-11 18:1, cis-9, trans-11 CLA, trans-11, trans-13 CLA, and cis-9 18:1.

Overall Chilliard et al (2009) study showed an increase in milk yield with linseed oil supplementation and a decrease with extruded linseeds. They hypothesized that effects on milk yield could be explained by a decrease in dry matter intake and fiber digestibility because there is a high level of oil or extruded linseed intake. The positive effects of feeding CLS on dry matter intake and milk yield are consistent with the studies done previously on ground crude linseed. Because whole crude linseed (CLS diet) does not release FA in the rumen fluid as fast as the ELS and LSO, the ruminal function was not disrupted (Chilliard et al., 2009). In the ELS and LSO supplementation diets the low milk fat content and yield could be due to a slower rate of mammary lipogenesis as a result of the addition of polyunsaturated oil to the starch diet (Chilliard et al., 2009). The fact that the CLS diet did not decrease milk fat content or yield was consistent with previous studies.

Beauchemin et al. (2008) conducted a study on crushed sunflower, flax, or canola seeds in lactating dairy cow diets and the effects they had on CH₄ production, ruminal fermentation, and milk production. Their goal was to reduce CH₄ production by adding long-chain FA that had different amounts of saturation and rumen availability (Beauchemin et al., 2008). Sixteen lactating cows were used, in a crossover design that split the cows into two groups and they were fed 4 dietary treatments in 28 d periods. The study included 8 primiparous cows that were all ready ruminally cannulated, and 8 multiparous cows that were not cannulated. The treatments were calcium salts of long-chain FA (CTL), crushed sunflower seeds (SS), crushed flax seed (FS), and crushed canola seed (CS). The CTL was used as the control diet because it was assumed that it would have no effect on the rumen fermentation or CH₄ production, and each diet was formulated to provide each cow with 3.3% added fat (Beauchemin et al., 2008).

Their results (Beauchemin et al., 2008) showed that the CLS contained less fat than had been expected and the oilseeds were higher in fat than had been expected. Therefore, the average amount of fat added was approximately 3.7% of dietary dry matter and total fat content averaged 6.5%. Only the CS diet had a similar digestible dry matter compared to the CTL. SS and CS diets showed an increase in dry matter intake but a decrease

in nutrient digestibility. All three oilseed treatments lowered CH₄ production. The CH₄ production was expressed as a percent of gross energy intake and was not changed by the SS diet but was 20% lower for the FS and 17.5% lower for the CS when compared to the CTL diet. However, the CS diet was the only diet to lower CH₄ production when expressed on the basis of digestible dry matter intake.

Overall the study looked at reducing enteric CH₄ emissions from lactating dairy cows by adding oilseed to the diet to supply long-chain FA. Feeding oilseeds proved to reduce the enteric CH₄ emissions (g/d) by an average 13% (Beauchemin et al., 2008). When looking at the individual oilseeds the SS was less effective at reducing the CH₄ production than the FS and the CS. With previous studies a concern has been that the decrease in the CH₄ production could be due to a decreased dry matter intake, which would lead to decreased milk production. In this study the effect of SS on lowering the emissions of CH₄ was due to a reduction in digestibility of dry matter intake (Beauchemin et al., 2008). For the FS treatment, the decrease was partially due to a decrease in digestible dry matter intake but in the CS diet none of the reduction effects were attributed to the effects on digestibility or intake (Beauchemin et al., 2008). Therefore, adding oilseeds

to the diet can help to lower CH₄ emissions. However, some oilseeds reduce CH₄ at the expense of diet digestibility (Beauchemin et al., 2008).

The above-cited results are summaries of two relevant studies conducted with dairy cows fed flaxseed/linseed. The study done at the UNH ODRF involved supplementing increasing levels of flaxseed and effects on milk production, FA profile, fecal and urinary outputs, ruminal fermentation, enteric methane emissions, and blood parameters. For the purpose of this thesis, only animal performance, ruminal fermentation, and enteric CH₄ emissions are shown.

Hypothesis

We hypothesized that feeding ground flaxseed will maintain the milk omega-3 fatty acids in CLA as the cows transitioned into winter from a heavily pasture-based diet to a stored forage and concentrate diet and decrease enteric methane emissions in organic dairy cows.

Methodology

Twenty lactating Jersey cows from the UNH ODRF were selected and supplemented increasing levels of flaxseed over a period of three months (Late-February to May). The treatment was in addition to their grass-legume-based diet. The treatments were added in random sequences in five replicated 4 x 4 Latin Squares. The treatments were (% of diet dry matter):

0, 5, 10, or 15% ground flaxseed. Each period lasted 21 days, with the first 14 days for diet adaptation and 7 days for collecting samples and analyzing data.

During the winter months of the study, the cows were held in a bedded-pack barn with access to feed via Calan doors (American Calan, Northwood, NH). Each cow wore a transponder that only opened one specific Calan door to access her feed, which allowed for precise individual measurement of feed consumption and refusals. Each day the refusals were collected and the amount fed to each cow was adjusted. The goal was to have the refusal within 5 to 10% of the total dry matter intake. The feed was also sampled daily during the final week of each treatment rotation to analyze its nutrient composition. The total mixed ration contained 65% grass-legume baleage plus one of the following supplements: 1) 0% ground flaxseed, 27% corn meal, and 8% soybean meal, 2) 5% ground flaxseed, 24% corn meal, and 6% soybean meal, 3) 10% ground flaxseed, 21% corn meal, and 4% soybean meal, or 4) 15% ground flaxseed, 17.5% corn meal, and 2.5% soybean meal.

Prior to the start of the study, body weights of the cows were measured during three consecutive days. Then the measurements were repeated during the last three days of each period. Throughout the study the

cows were milked twice per day. The milk yield was recorded at each milking and four samples (a.m. and p.m. milkings for two consecutive days) per cow were collected. The milk samples were then analyzed for the following components: fat, protein, lactose and milk urea nitrogen using mid-infrared spectrophotometry (Dairy One Cooperative Inc., Ithaca, NY). Energy corrected milk and fat corrected milk was estimated according to Orth (1992) and the NRC (2001) respectively. Blood samples were also collected from the coccygeal artery or vein four hours after morning feeding on the last two days of each period to measure levels of urea nitrogen and nonesterified FA using commercial available kits. The feces and urine samples were collected twice daily over three consecutive days of each period so the nutrient digestibility and microbial protein synthesis could be determined. Methane was collected using the sulfur hexafluoride technique as outlined by Johnson and Johnson (1995), and analyzed with gas chromatography.

Results

The following table (Table 1: Effects of different dietary levels of ground flaxseed on cow performance, milk components, blood composition and methane emissions from lactating organic dairy cows.) highlights the effects

of increasing dietary levels of ground flaxseed on cow performance, milk components, and CH₄ emissions from lactating organic dairy cows.)

Table 1: Effects of different dietary levels of ground flaxseed on cow performance, milk components, blood composition and methane emissions from lactating organic dairy cows.

	Dietary Levels of Ground Flaxseed (% Dry Matter)				² SED	Contrasts (P-Value)	
	0	5	10	15		Linear	Quadratic
³ DMI, kg/d	16.9	16.8	15.7	16.1	0.29	<0.001	0.33
Milk yield, kg/d	21.2	21.0	20.6	19.9	0.35	<0.001	0.25
4% fat-corrected milk, kg/d	23.4	23.6	22.4	22.0	0.64	0.01	0.51
Energy-corrected milk, kg/d	25.1	25.2	24.0	23.4	0.66	<0.01	0.45
Milk fat, %	4.59	4.58	4.62	4.65	0.10	0.49	0.78
Milk fat, kg/d	0.98	0.99	0.94	0.93	0.031	0.05	0.69
Milk protein, %	3.44	3.39	3.37	3.32	0.026	<0.0001	0.97
Milk protein, kg/d	0.73	0.73	0.69	0.66	0.019	<0.001	0.33
Milk lactose, %	4.80	4.79	4.79	4.84	0.049	0.46	0.36
Milk lactose, kg/d	1.04	1.05	0.99	0.98	0.027	<0.01	0.58
⁴ MUN, mg/dL	12.8	12.7	12.0	10.9	0.36	<0.0001	0.06
Methane, g/d	274	250	222	190	29.2	<0.01	0.85
Total VFA, mM	64.9	65.4	66.2	71.3	4.00	0.13	0.42
Acetate, mol/100 mol	72.8	72.3	72.2	71.8	0.29	<0.01	0.73
Propionate, mol/100 mol	14.4	14.7	15.4	16.4	0.25	<0.001	0.06
Butyrate, mol/100 mol	10.4	10.6	10.2	9.72	0.19	<0.001	<0.01

¹Significance was declared a $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$; ²SED = standard error of least square means difference; ³DMI = dry matter intake; ⁴MUN = milk urea nitrogen.

The data that has been analyzed so far has shown to have many components that are significant regarding the milk production and milk components with supplementing ground flaxseed. DMI decreased at a linear rate ($P < 0.001$) from 0 to 15% ground flaxseed. Shown in figure 1 below.

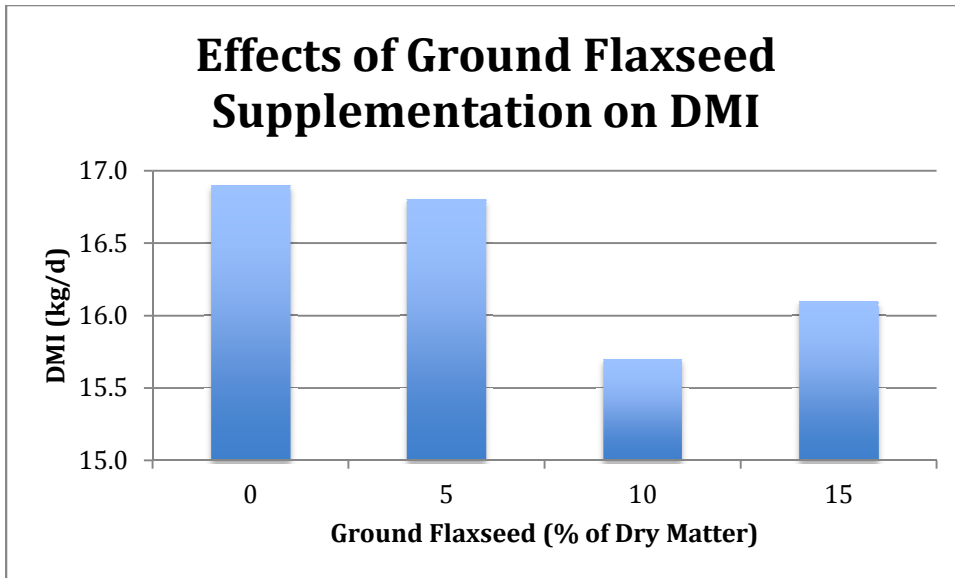


Figure 1: DMI with increasing amounts of ground flax seed.

Linear (P<0.001)
 Quadratic (P=.33)
 SED = .29

Milk yield also decreased at a linear rate ($P < 0.001$). Shown in figure 2 below.

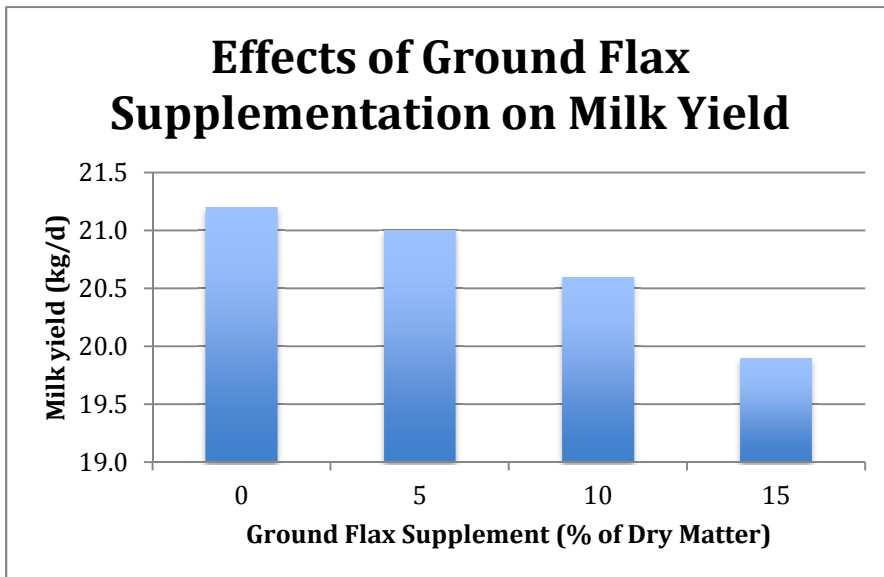


Figure 2: Milk Yield with increasing amounts of ground flax seed.

Linear (P<0.001)
 Quadratic (P=.25)
 SED = .35

Yields of milk fat, protein, and lactose also decreased at a linear rate, with the following P -values of 0.05, <0.001, <0.01 respectively. Shown in figure 3 below.

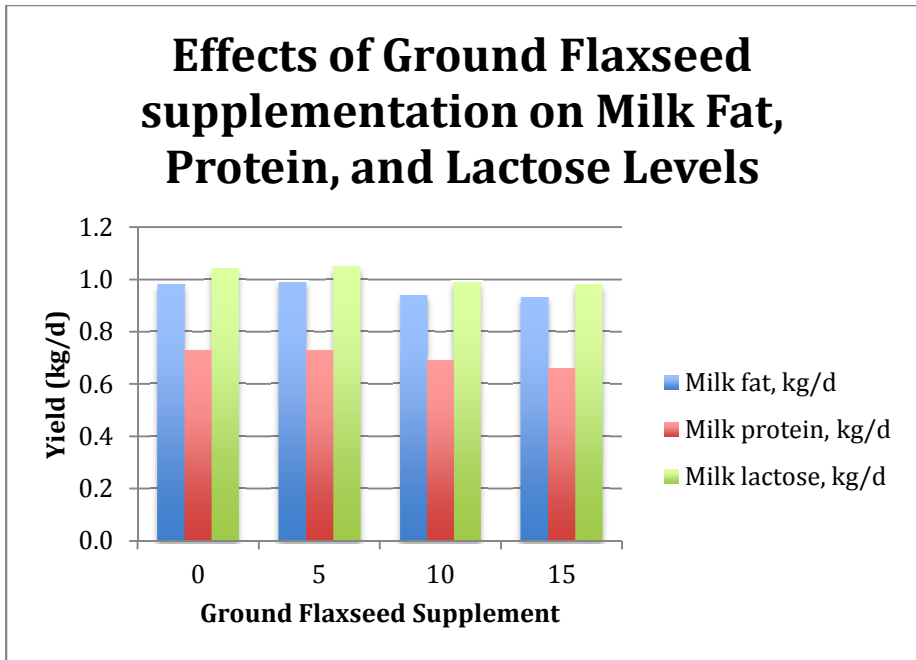


Figure 3: Milk fat, protein, and lactose with increasing amounts of ground flax seed.

Fat:
 Linear (P=.05)
 Quadratic (P=.69)
 SED = 0.031

Protein:
 Linear (P<0.0001)
 Quadratic (P=.33)
 SED = 0.049

Lactose:
 Linear (P<0.01)
 Quadratic (P=0.06)
 SED = .36

Methane production was decreased at a linear rate as well ($P < 0.001$) from 0 to 15% of dry matter being ground flaxseed. Shown in figure 4 below.

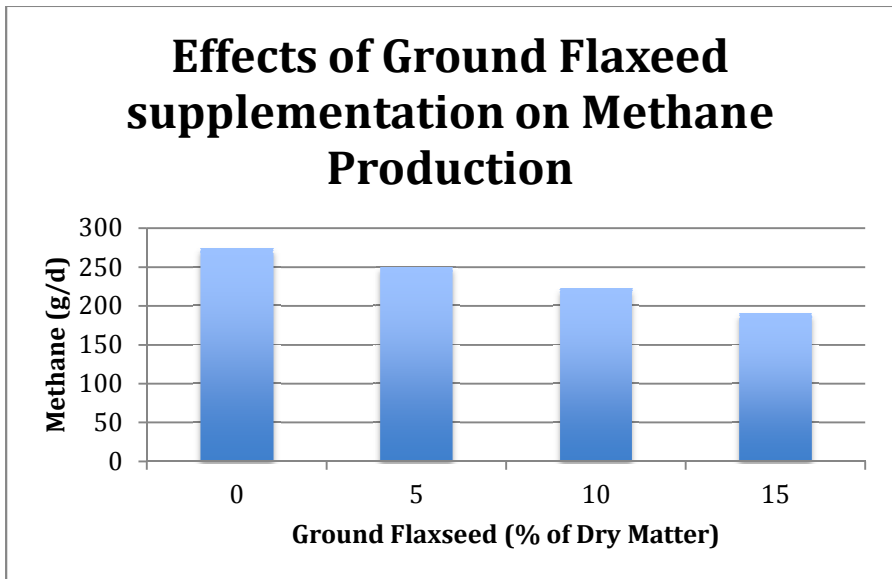


Figure 4: Methane Production with increasing amounts of ground flax seed.

Linear (P<0.001)
 Quadratic (P=.85)
 SED = 29.2

The molar proportion of acetate ($P < 0.01$) and butyrate ($P < 0.001$) were also decreased at a linear rate respectively, and propionate was increased at a linear rate ($P < 0.001$). Shown below in figure 5.

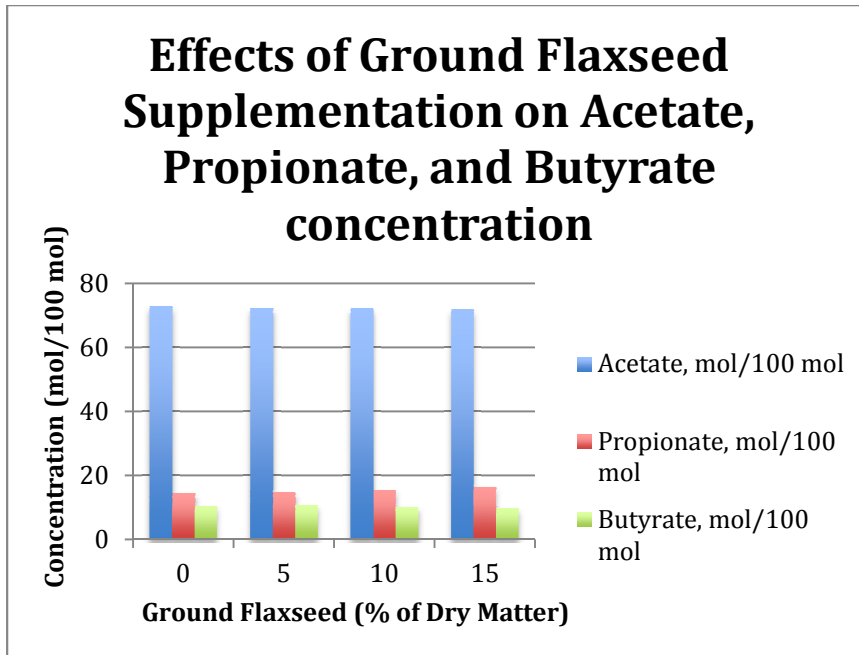


Figure 5: Acetate, Butyrate, and Propionate concentration with increasing amounts of ground flax seed.

Acetate:
 Linear (P<0.01)
 Quadratic (P=.73)
 SED = 0.29

Propionate:
 Linear (P<0.001)
 Quadratic (P=.06)
 SED = 0.25

Butyrate:
 Linear (P<0.001)
 Quadratic (P<0.01)
 SED = .19

Discussion and Implications

So far, ground flaxseed is showing to have significant effects on the milk yield, milk composition, CH₄ production, and the concentration of the individual volatile fatty acids, in this short-term, change-over design study. However, research is needed to determine the long-term effects of ground flaxseed on milk yield, milk composition, and milk health.

With the results from this study, it does appear that ground flaxseed decreases dry matter intake when supplemented to lactating dairy cows. Therefore the subsequent decrease in milk yield is expected due to the decreased dry matter intake. The linear decreases in yields of milk fat, milk protein, and milk lactose followed the linear decrease in milk yield.

The decrease in CH₄ production was also reduced linearly and agreed with our hypothesis to mitigate CH₄ production. This could be due to the flax but also could be a result of the decrease in dry matter intake.

The amount of acetate, propionate, and butyrate were also results that were noteworthy from this study. The acetate and butyrate concentration decreased linearly and the propionate concentration increased linearly. Acetate and butyrate are volatile FA that are produced by cellulolytic bacteria. Cellulolytic bacteria are affected by the concentration of FA that are added or decreased in the rumen due to the toxicity effect that the FA has on the cellulolytic bacteria. The linear increase in propionate may be explained by hydrogen being used to synthesize propionate rather than CH₄.

Overall, the data that has been analyzed so far has proven to have significant effects on lactating dairy cows milk yield and milk components along with the volatile fatty acid concentration and the CH₄ output.

In the upcoming months the milk will be analyzed to look at the FA composition, and the body condition score, among other variables. This data will provide further insight as to if ground flaxseed is effective to feed to lactating dairy cows. So far, it looks to have a negative effect when fed at 10 and 15% of the dry matter. More research needs to be done to determine the

long-term effects of ground flax seed, if the processing of the feedstuff has an effect, or if a different lipid would work more efficiently.

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