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Increasing the concentration of linolenic acid in diets fed to Jersey cows in late lactation does not affect methane production

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ABSTRACT

Although the inclusion of fat has reduced methane production in ruminants, relatively little research has been conducted comparing the effects of source and profile of fatty acids on methane production in lactating dairy cows. A study using 8 multiparous (325 ± 17 DIM; mean ± SD) lactating Jersey cows was conducted to determine effects of feeding canola meal and lard versus extruded byproduct containing flaxseed as a high-C18:3 fat source on methane production and diet digestibility in late-lactation dairy cows. A crossover design with 32-d periods (28-d adaptation and 4-d collections) was used to compare 2 different fat sources. Diets contained approximately 50% forage mixture of corn silage, alfalfa hay, and brome hay; the concentrate mixture changed between diets to include either (1) a conventional diet of corn, soybean meal, and canola meal with lard (control) or (2) a conventional diet of corn and soybean meal with an extruded byproduct containing flaxseed (EXF) as the fat source. Diets were balanced to decrease corn, lard, and canola meal and replace them with soybean mean and EXF to increase the concentration of C18:3 (0.14 vs. 1.20% of DM). Methane production was measured using headbox-style indirect calorimeters. Cattle were restricted to 95% ad libitum feed intake during collections. Milk production (17.4 ± 1.04 kg/d) and dry matter intake (15.4 ± 0.71 kg/d) were similar among treatments. Milk fat (5.88 ± 0.25%) and protein (4.08 ± 0.14%) were not affected by treatment. For methane production, no difference was observed for total production (352.0 vs. 349.8 ± 16.43 L/d for control vs. EXF, respectively). Methane production per unit of dry matter intake was not affected and averaged 23.1 ± 0.57 L/kg. Similarly, methane production per unit of energy-corrected milk was not affected by fat source and averaged 15.5 ± 0.68 L/kg. Heat production was similar, averaging 21.1 ± 1.02 Mcal/d. Digestibility of organic matter, neutral detergent fiber, and crude protein was not affected by diet and averaged 69.9, 53.6, and 73.3%, respectively. Results indicated that increasing C18:3 may not affect methane production or digestibility of the diet in lactating dairy cows.

Key words: linolenic acid, methane, milk

INTRODUCTION

The Innovation Center for the US Dairy (2014) set a goal for the US dairy industry to lower the total greenhouse gas production by 25% by 2020. High-producing dairy cattle produce approximately 630 L of methane (CH_4) each day (Hristov et al., 2013), and this is affected by diet composition, feed intake, and digestibility (Hristov et al., 2018). Methane is of major interest because its effect on global warming is approximately 21 to 25 times more potent than that of carbon dioxide (CO_2). One strategy believed to reduce CH_4 production in cattle is to add supplemental fat to the diet (Knapp et al., 2014). In support of this, research has demonstrated that the inclusion of fat reduced CH_4 production without adversely affecting milk production or milk components (Beauchemin et al., 2009). Although the reason for this effect has not been clearly determined, it has been suggested that this fat was toxic to CH_4-producing rumen microbes or that the oil provided an alternative hydrogen sink and that rather than producing CH_4, rumen microbes acted to hydrogenate fatty acids (Nagaraja et al., 1997; Martin et al., 2010; Knapp et al., 2014).

The amount of fat included and even the fatty acid profile of those fats has been shown to reduce CH_4 production in ruminants (Martin et al., 2010). This is thought to occur through 3 interwoven and perhaps

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even independent factors, which have been outlined by Beauchemin et al. (2009). The first is that supplementing fat in place of carbohydrates results in a reduction on overall rumen fermentation; doing so may also have a direct and negative effect on fiber digestion (Huhtanen et al., 2009; Knapp et al., 2014). Second, fat may have a direct effect on rumen methanogens. Third, supplementation can increase the extent of biohydrogenation, thereby acting as a sink for hydrogen (Blaxter and Czerkawski, 1966; Patra et al., 2017); however, the capacity of this route to reduce total CH_4 production has been suggested to be small (Jenkins et al., 2008). In general, compared with SFA, medium-chain fatty acids such as C12:0 and C14:0, C18:3, and other PUFA are more potent in reducing CH_4 (Patra et al., 2017). In vitro incubations of flaxseed and fish oil, which are rich in UFA, have been observed to reduce CH_4 production (Song et al., 2010; Soder et al., 2012). Additionally, using calcium salts of fish oil, which are high in n-3 fatty acids, Castañeda-Gutiérrez et al. (2007) observed extensive biohydrogenation of the n-3 fatty acids eicosapentaenoic acid (>85%) and docosahexaenoic acid (>75%). Even though biohydrogenation occurred at a high rate when feeding fish oil, DMI was decreased between 15 and 20%. Thus, feeding a source of linolenic acid may affect CH_4 production. Extruded flaxseed contains approximately 53% linolenic acid as a percentage of total fatty acid profile (NRC, 2001) and may prove beneficial when aiming to reduce enteric CH_4 production (Martin et al., 2010). In support of this, Benchaar et al. (2015) supplemented 4% flaxseed oil to lactating dairy cows consuming approximately 60% forage (either red clover or corn silage) and observed a 26% reduction in CH_4 production. Crude fat increased from 3.7 to 6.5% in the red clover treatments and increased from 2.3 to 5.8% in the corn silage treatments. When flaxseed oil was supplemented to diets containing corn silage, a 15% reduction in NDF digestibility and a 3% increase in CP digestibility were observed. The differing fat concentration may have led to different fatty acid profiles in these treatments; however, specific fatty acid profiles were not reported. Consequently, it is not known whether the observed effect of flaxseed oil was a result of simply fat or a unique effect of linolenic acid (Beauchemin et al., 2009). Thus, in vivo research is needed to compare diets that have similar concentrations of fat but differ in the proportions of linolenic acid in that fat. Therefore, our objective was to determine the effects of increasing the concentration of linolenic acid in diets with similar concentrations of fat when fed to lactating dairy cattle. We hypothesized that increased supplementation of linolenic acid would reduce enteric CH_4 production in lactating dairy cows without affecting milk production, milk composition, and diet digestibility.

MATERIALS AND METHODS

All animal care and experimental procedures were approved by the University of Nebraska–Lincoln Animal Care and Use Committee. Eight multiparous lactating Jersey cows (325 ± 17 DIM; mean ± SD) with a BW averaging 485.5 ± 19.6 kg were used. These cows had been used previously in a nutrition study (Drehmel et al., 2018) and were acclimatized to all animal procedures involving indirect calorimeters. All cows were housed in a temperature-controlled barn at the Dairy Metabolism Facility at the Animal Science Complex at the University of Nebraska–Lincoln and milked at 0700 and 1800 h in individual tiestalls equipped with rubber mats.

The experimental design was a crossover design. Cows were randomly assigned to 1 of 2 dietary treatments: (1) a conventional diet of corn, soybean meal, and canola meal with lard (**CON**) or (2) a conventional diet of corn and soybean meal with an extruded byproduct containing flaxseed (**EXF**; O&T Farms, Regina, SK, Canada) as the source of linolenic acid (Table 1). This was a byproduct of a single-screw extrusion process. Treatments alternated over 2 experimental periods, and measurements were collected on each animal consuming each treatment. The study was conducted with a total of 2 experimental periods, each being 32 d in duration. Each period included 28 d for ad libitum diet adaptation, targeting about 5% refusals during that time, followed by 4 d of collection with 95% ad libitum feeding to reduce the amount of refusals.

The experimental diets and associated concentrate mixes contained similar concentrations of CP and crude fat, but the diets differed in fatty acid profile. The fatty acid profile was altered in the EXF diet by completely replacing lard and partially replacing canola meal with 10.5% of an extruded byproduct containing flaxseed. The concentration of linolenic acid in the control diet was 3.51 ± 0.239% of the total fatty acids (0.14 ± 0.02% of DM), whereas that of the EXF treatment was 24.5 ± 0.2116% of the total fatty acids (1.20 ± 0.20% of DM). The proportion of forage remained constant across treatments. The Cornell-Penn-Miner Dairy model (Boston et al., 2000) was used to balance diets. All dietary treatments contained corn silage, alfalfa hay, brome hay, and a concentrate mixture (comprising a mixture of all ingredients except forages), which were combined as a TMR. The TMR was mixed in a Calan Data Ranger (American Calan Inc., Northwood, NH) and fed to the cows once daily at 1000 h.

Laboratory Analysis

Individual feed ingredients were sampled (500 g) on the first day of each collection period and dried at 60°C for 48 h and ground to pass through a 2-mm screen using a Wiley mill (Arthur A. Thomas Co., Philadelphia, PA). A subsample was then sent to Cumberland Valley Analytical Services Inc. (Hagerstown, MD) for analysis of DM (AOAC International, 2000), N (Leco FP-528 N Combustion Analyzer, Leco Corp., St. Joseph, MO), NDF with sodium sulfite (Van Soest et al., 1991), ADF (method 973.18; AOAC International, 2000), lignin (Goering and Van Soest, 1970), NFC [100 − (% NDF + % CP + % fat + % ash)], sugar (DuBois et al., 1956), starch (Hall, 2009), crude fat (method 2003.05; AOAC International, 2006), ash (method 943.05; AOAC International, 2000), and minerals (method 985.01; AOAC International, 2000). Total mixed rations were sampled (500 g) on each day of each collection period and were frozen at −20°C. The samples were composited by period and treatment. A subsample was sent to Cumberland Valley Analytical Services Inc. (Waynesboro, PA) for complete nutrient analysis with the same laboratory processes as the individual feed ingredients. Particle size of the TMR was determined according to Heinrichs and Kononoff (2002) using the Penn State Particle Separator. Each day of the collection period, refusals were sampled and frozen at −20°C. The samples were composited by period and individual cow. A subsample was sent to Cumberland Valley Analytical Services Inc. for analysis of DM, N, NDF with sodium sulfite, starch, and ash using previously referenced methods. Samples of TMR and feces were also analyzed for fatty acids by GC with flame ionization detector after direct methylation on composite TMR samples using C13:0 or C17:1 (NuChek Prep Inc., Elysian, MN) as internal standards as described by Rico et al. (2014).

Total fecal output was collected from each individual cow during the collection period for 4 consecutive days. A 137-cm × 76-cm rubber mat (Snake River Supply, Idaho Falls, ID) was placed behind the cow to collect feces. Urine was not collected in this study; however, a catheter was placed in each cow before the experiment so urine flowed away from the feces and thus the two were never mixed. The feces were deposited multiple times a day from the rubber mats into a large garbage container (Rubbermaid, Wooster, OH) with a black garbage bag covering the top to reduce N losses before subsampling. The feces were subsampled (4% wet basis) every day for 4 consecutive days and dried at 60°C in a forced-air oven for 48 h and then composited by cow and period before being ground to pass through a 2-mm screen (Wiley mill, Arthur H. Thomas Co., Philadelphia, PA). The ground feces samples were sent to Cumberland Valley Analytical Services Inc. for nutrient analysis of DM, N, NDF with sodium sulfide, starch, and ash using previously referenced methods.

Milk production was measured daily, and milk samples were collected during both the morning and afternoon milking times for 4 consecutive days, or d 29 to 32 of the entire period. A sample from each cow at each milking was placed in a 50-mL conical tube; this was preserved using 2-bromo-2-nitropropane-1,3 diol and sent to Heart of America DHIA (Kansas City, MO), where it was analyzed for fat, protein, lactose, SNF, MUN, and SCC using a Bentley FTS/FCM Infrared Analyzer (Bentley Instruments, Chaska, MN). To determine the DM content of individual feed ingredients, TMR, refusals, and feces samples were dried at 60°C in a forced-air oven for 48 h and then composited by treatment or cow and period. Feed ingredients, refusals,

Table 1. Diet composition (% of DM) of control (CON) and extruded byproduct containing flaxseed (EXF) treatments fed to lactating Jersey cows in late lactation averaging 325 ± 17 DIM[1]

Item	Treatment	
	CON	EXF
Corn silage	27.5	27.5
Alfalfa hay	21.0	21.0
Brome hay	1.57	1.57
Ground corn	20.2	17.3
Soybean meal	5.53	6.28
Extruded byproduct containing flaxseed[2]	0.00	10.5
Canola meal	9.17	2.62
Non-enzymatically browned soybean meal[3]	5.24	5.24
Ground soybean hulls	5.24	5.24
Lard	1.78	0.00
Calcium carbonate	0.81	0.81
Sodium bicarbonate	0.67	0.67
Calcium salts of LCFA[4]	0.59	0.59
Blood meal	0.26	0.26
Magnesium oxide	0.26	0.26
Salt	0.20	0.20
Vitamin premix[5]	0.04	0.04
Trace mineral premix[6]	0.04	0.04
ME,[6] Mcal/kg	2.69	2.68
NE_L,[6] Mcal/kg	1.74	1.73

[1]For each treatment, a concentrate grain mixture was included in the TMR; this mixture comprised all ingredients listed except forages.

[2]Marketed as Linpro-R by O&T Farms (Regina, SK, Canada). Composition: DM 94.0%, CP 22.0% of DM, soluble protein 33.3% of DM, acid detergent insoluble CP 1.01% of DM, neutral detergent insoluble CP 3.79% of DM, crude fat 21.3% of DM, ADF 15.3% of DM, NDF 27.1% of DM, NFC 32.0% of DM, starch 17.5% of DM, sugar 4.06% of DM, lignin 3.32% of DM, ash 4.49% of DM.

[3]Soypass (LignoTech, Overland Park, KS).

[4]Calcium salts of long-chain fatty acids marketed as Megalac by Church & Dwight Co. Inc. (Princeton, NJ).

[5]Formulated to supply approximately 2,300 mg/kg of Co, 25,000 mg/kg of Cu, 2,600 mg/kg of I, 1,000 mg/kg of Fe, 150,000 mg/kg of Mn, 820 mg/kg of Se, and 180,000 mg/kg of Zn in total rations.

[6]Values calculated at time of formulation using the Cornell-Penn-Miner dairy model (Boston et al., 2000).

and feces were ground as previously described for feces and corrected for laboratory DM.

Heat production was determined through the headbox-style indirect calorimeters described by Foth et al. (2015) and Freetly et al. (2006) in a temperature-controlled barn. For each cow, gas was collected during 2 consecutive 23-h intervals. Oxygen (O_2) consumption as well as CO_2 and CH_4 production were measured each day. An average of the gas production for the 2 consecutive days was taken, with minimal variation observed between days. The design of the headboxes allowed for feed to be placed in the bottom of the box, and ad libitum access to water was available for the cows from a water bowl placed inside the headbox. Within the headbox, temperature and dew point were recorded every minute for a 23-h interval using a probe (model TRH-100, Pace Scientific Inc., Moorseville, NC) that was connected to a data logger (model XR440, Pace Scientific Inc.). Fifteen minutes before the start of the collection, the doors were closed and the motor was turned on to allow for several air turnovers before gases were collected. Line pressure was measured using a manometer (item no. 1221-8, United Instruments, Westbury, NY). Barometric pressure of the room was also recorded using a barometer (Chaney Instruments Co., Lake Geneva, WI) and uncorrected for sea level. Total volume of gas that passed through the headbox during each run was measured using a dry gas meter (model AL425, American Meter, Horsham, PA). From the headbox, continuous amounts of outgoing and incoming air were diverted to 2 different collection bags (61 cm × 61 cm, LAM-JAPCON-NSE, 44 L; PMC, Oak Park, IL) using glass tube rotameters (model 1350E Sho-Rate "50," Brooks Instruments, Hatfield, PA). Collection bags with gas samples inside were analyzed (Emerson X-stream 3-channel analyzer, Solon, OH) at the US Meat Animal Research Center according to Nienaber and Maddy (1985). Measurements collected from the 2 d were averaged to obtain a single observation. Heat production was estimated through calculation of O_2 consumption and CO_2 and CH_4 production without correction for urinary N loss according to Nienaber and Maddy (1985; Equation 1). The gaseous products were reported in liters; respiratory quotient was calculated using the ratio of CO_2 produced to O_2 consumed and was not corrected for N:

$$\text{heat production (Mcal/d)} = (16.18 \times O_2\ \text{L} + 5.02 \times CO_2\ \text{L} - 2.17 \times CH_4\ \text{L})/4.183. \quad [1]$$

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Treatment and period were modeled as fixed effects, and cow was modeled as a random effect. The LSMEANS option was used to generate least squares means of treatments listed in this study. Significance was declared at $P \leq 0.05$, and trends were declared at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Diet Composition

When UFA are fed to cattle, lipolysis in the rumen occurs at a high rate followed by biohydrogenation, which does not always occur completely or at a constant rate (Beam et al., 2000). In an in vitro study, Beam et al. (2000) observed the losses of UFA to occur at 78, 83, and 94% of their intake for oleic, linoleic, and linolenic acid, respectively. The ingredient composition of diets was manipulated to increase the concentration of linolenic acid and is presented in Table 1. Chemical compositions for feed ingredients and TMR are presented in Tables 2, 3, and 4. By design, chemical composition was similar between diets, except for fatty acid profile; most notable was that C18:3 was greatest in the EXF treatment (3.5 vs. 24.5% of total fatty acids for CON vs. EXF, respectively).

DMI and Milk Production and Composition

Although some fats (e.g., those from fish oil) may influence DMI, no difference was expected between treatments in the current study. Dry matter intake was not different ($P = 0.262$) between treatments and averaged 15.4 ± 0.71 kg/d (Table 5). Similarly, in lactating dairy cows, Martin et al. (2016) observed no difference in DMI with extruded flaxseed treatments that increased fat from about 2.5 to about 7.8% of dietary DM. In the current study, similar DMI among treatments may be the result of similar concentrations of crude fat in the diet. However, Martin et al. (2008) observed decreased DMI with extruded flaxseed supplementation in lactating dairy cattle with fatty acids increasing from 2.6 to 7.4%. Neither milk fat percentage nor yield were different ($P = 0.864$ and 0.512, respectively), averaging 5.88 ± 0.25% and 1.02 ± 0.09 kg/d for milk fat percentage and yield, respectively. Beauchemin et al. (2009) tested the effect of including crushed flaxseed in replacement of calcium salts of long-chain fatty acids and beet pulp and observed no difference in milk fat production. In the current study, linolenic acid from extruded flaxseed likely was not included at a great enough concentration to induce milk fat depression. Similar to milk fat, ECM was not different ($P = 0.446$) among treatments, with an average of 23.9 ± 1.84 kg/d. Milk protein percentage and yield were not different ($P = 0.694$ and

Table 2. Chemical composition (% of DM unless noted) of diets for control (CON) and extruded byproduct containing flaxseed (EXF) treatments[1]

	CON		EXF	
Chemical composition	Mean	SD	Mean	SD
DM, %	62.1	0.21	61.9	0.92
Ash	7.92	0.04	7.92	0.03
CP	18.2	1.72	18.2	0.87
Soluble protein	5.90	0.42	5.63	0.96
Acid detergent insoluble CP[2]	1.13	0.19	0.96	0.01
Neutral detergent insoluble CP[2]	2.39	0.14	2.22	0.11
ADF	21.3	1.35	21.8	1.92
NDF	32.8	0.20	33.3	2.27
Lignin	4.16	0.78	4.18	0.97
NFC	39.0	2.44	38.0	0.78
Starch	23.5	0.23	23.4	0.69
Sugar	3.81	0.39	3.7	0.64
Crude fat	4.50	0.63	4.87	0.50

[1]Determined by Cumberland Valley Analytical Services (Waynesboro, PA).

[2]Determined by Penn State University (University Park, PA; Rico et al., 2014).

0.334, respectively) by treatment, averaging of 4.08 ± 0.14% and 0.70 ± 0.05 kg/d for milk protein percentage and yield, respectively. These data are consistent with previous research in lactating dairy cattle consuming extruded flaxseed, where both Martin et al. (2008) and Beauchemin et al. (2009) observed no difference in milk protein percentage or yield. In the current study, dietary CP was high and thus the supply of MP was not expected to limit milk protein for the late-lactation dairy cows.

Gas Consumption and Production

Oxygen consumption and CO_2 production were not different ($P = 0.960$ and 0.959, respectively) between treatments, averaging 4,137.4 ± 205.1 and 4,351.4 ± 200.6 L/d for O_2 and CO_2, respectively (Table 6). The respiratory quotient was not different ($P = 0.413$) between the control and extruded flaxseed, with a mean of 1.06 ± 0.01, indicating that the cows were in a positive energy balance. Similarly, estimated heat production and heat production per unit of metabolic BW were not different ($P = 0.980$ and 0.685, respectively), averaging 21.1 ± 1.02 Mcal/d and 215.1 ± 7.79 kcal/$BW^{0.75}$, respectively. Feeding extruded flaxseed has been reported to decrease daily CH_4 production by 38 to 70% (Martin et al., 2008, 2016). However, Martin et al. (2008) increased crude fat in the flaxseed diet from 2.6% to approximately 7.4% of dietary DM. Similarly, Martin et al. (2016) increased concentration of crude fat in the treatments containing extruded flaxseed from about 2.5% to about 7.8% of dietary DM compared with the control. In the current study, crude fat was

Table 3. Chemical composition[1] (% of DM unless noted) of alfalfa hay, corn silage, brome hay, control concentrate (CON CONC), and extruded byproduct containing flaxseed concentrate (EXF CONC) used to make the TMR fed to lactating Jersey cows in late lactation

	Alfalfa		Corn silage		Brome hay		CON CONC		EXF CONC	
Chemical composition	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM, %	86.8	1.20	32.8	2.40	87.1	1.56	88.9	0.28	89.5	0.64
CP	19.6	2.83	7.60	0.42	9.15	0.49	23.8	2.47	23.7	0.78
Soluble protein	12.0	7.28	4.05	0.35	2.35	0.35	4.50	3.68	3.95	0.92
Acid detergent insoluble CP	1.81	0.08	0.79	0.05	1.10	0.01	1.04	0.33	0.70	0.05
Neutral detergent insoluble CP	2.86	0.24	0.79	0.06	3.78	0.23	3.03	0.15	2.68	0.35
ADF	35.9	3.11	26.2	0.49	41.0	0.78	11.8	1.70	12.8	2.83
NDF	44.7	2.12	40.4	0.42	65.5	0.85	22.5	1.56	23.5	3.39
Lignin	7.94	0.49	3.75	0.16	5.69	0.00	2.75	1.44	2.80	1.82
NFC[2]	25.9	1.41	43.7	0.21	16.3	0.35	42.7	4.38	40.7	2.05
Starch	1.35	0.07	32.4	0.00	0.85	0.07	28.5	0.42	28.4	1.34
Sugar	4.80	0.14	0.55	0.21	6.55	0.78	5.10	0.71	4.95	1.20
Crude fat	1.87	0.60	3.69	0.78	2.46	0.30	6.11	0.57	6.86	0.30
Ash	10.9	0.37	5.43	0.05	10.2	0.59	8.00	0.06	7.99	0.08
Ca	1.28	0.11	0.19	0.01	0.46	0.05	1.49	0.20	1.13	0.15
P	0.38	0.01	0.23	0.01	0.28	0.01	0.54	0.03	0.48	0.02
Mg	0.26	0.02	0.13	0.01	0.14	0.01	0.56	0.00	0.47	0.07
K	3.46	0.08	0.95	0.11	2.03	0.05	1.17	0.01	1.23	0.02
S	0.24	0.04	0.13	0.00	0.19	0.02	0.34	0.01	0.28	0.01
Na	0.03	0.00	0.02	0.01	0.02	0.00	0.59	0.03	0.41	0.10
Cl	0.11	0.01	0.08	0.01	0.27	0.03	0.30	0.02	0.26	0.11
Fe, mg/kg	291	69.3	165	19.1	189	51.6	281	9.19	451	185
Zn, mg/kg	25.5	0.71	21.0	4.24	20.5	2.12	196.0	8.49	187.0	113.1
Cu, mg/kg	8.50	0.71	5.50	0.71	7.00	0.00	29.0	4.24	27.0	9.90
Mn, mg/kg	41.5	4.95	32.5	7.78	47.0	2.83	129	16.3	97.5	14.8

[1]Values determined by Cumberland Valley Analytical Services (Waynesboro, PA).

[2]Calculated as 100 − (% NDF + % CP + % fat + % ash).

Table 4. Particle size distribution[1] and fatty acid profile of control (CON) and extruded byproduct containing flaxseed (EXF) diets

	CON		EXF	
Item	Mean	SD	Mean	SD
Particle size, mm				
>19.0	3.50	0.58	4.00	0.82
19.0–8.0	20.5	4.36	20.5	4.44
8.0–1.18	52.0	2.16	51.5	2.65
<1.18	24.0	2.94	23.5	3.51
Total fatty acid, % of DM	4.02	0.15	4.89	0.40
Profile, % of total fatty acids				
C14:0	1.146	0.102	0.319	0.017
cis-9 C14:1	0.178	0.018	0.008	0.011
C15:0	0.221	0.028	0.080	0.007
C16:0	21.08	0.527	14.79	0.820
cis-9 C16:1	1.121	0.106	0.218	0.003
C17:0	0.526	0.073	0.158	0.003
cis-10 C17:1	0.000	0.000	0.000	0.000
C18:0	7.901	0.881	3.681	0.066
cis-9 C18:1	28.13	1.021	22.68	0.209
cis-11 C18:1	1.539	0.024	1.021	0.002
cis-9,*cis*-12 C18:2	25.08	1.511	25.96	0.604
C20:0	0.393	0.022	0.377	0.021
cis-6,*cis*-9,*cis*-12 C18:3	0.065	0.005	0.150	0.002
cis-9,*cis*-12,*cis*-15 C18:3	3.514	0.239	24.5	2.116
cis-11 C20:1	0.164	0.053	0.098	0.022
C20:2n-6	0.118	0.005	0.084	0.015
C22:0	0.000	0.000	0.000	0.000
cis-13 C22:1	0.000	0.000	0.000	0.000
C20:4n-6	0.009	0.013	0.000	0.000
cis-13,*cis*-16 C22:2	0.000	0.000	0.000	0.000
C24:0	0.354	0.176	0.349	0.232
C24:1n-9	0.000	0.000	0.000	0.000
Unknown	8.457	2.278	5.516	1.172

[1]Determined using the Penn State Particle Separator on a wet basis (Heinrichs and Kononoff, 2002).

Table 5. Dry matter intake, milk yield and composition, BW, and BCS of lactating Jersey cows in late lactation fed control (CON) or extruded byproduct containing flaxseed (EXF) diets

	Treatment			
Item	CON	EXF	SEM[1]	*P*-value
DMI, kg/d	15.0	15.7	0.71	0.262
Milk yield, kg/d	16.8	17.8	1.04	0.375
ECM[2]	23.2	24.6	1.84	0.446
Feed conversion[3]	1.52	1.57	0.08	0.550
Fat, %	5.89	5.86	0.25	0.864
Fat yield, kg/d	0.99	1.04	0.09	0.512
Protein, %	4.09	4.07	0.14	0.694
Protein yield, kg/d	0.68	0.72	0.05	0.334
Lactose, %	4.68	4.72	0.04	0.381
MUN, mg/dL	20.0	19.5	1.00	0.575
Water intake, L/d	73.4	72.1	4.50	0.770
BW, kg	484.5	486.5	19.6	0.615
BCS[4]	3.78	3.78	0.07	1.000

[1]Lowest standard error of treatment means is listed.

[2]Calculated as 0.327 × milk yield (kg) + 7.2 × protein (kg), adjusted for 3.5% fat and 3.2% total protein (DRMS, 2014).

[3]Calculated as ECM/DMI.

[4]On a 1-to-5 scale according to Wildman et al. (1982).

formulated for similar inclusion, with the proportion of linolenic acid in total fatty acids highest in the EXF, as we hypothesized that the increased concentration of linolenic acid would decrease CH_4 production. Contrary to this, CH_4 production was not different ($P = 0.904$) between the control and extruded flaxseed, with an average of 350.9 ± 16.4 L/d (Table 5). Likewise, Livingstone et al. (2015) observed no difference in CH_4 production with extruded flaxseed, although diets containing flaxseed contained 3.0% fatty acids, whereas diets not containing flaxseed contained 2.2% fatty acids. Additionally, in the current study, CH_4 per unit of DMI and ECM was not different ($P = 0.343$ and 0.303, respectively) between the control and EXF treatments, averaging 23.1 ± 0.57 L/kg per day and 15.5 ± 0.68 L/kg per day, respectively. Similarly, CH_4 per unit of digestible DM and NDF was not different ($P = 0.531$ and 0.397, respectively) between the control and EXF treatments, with an average of 34.3 ± 1.92 L/kg and 44.4 ± 4.23 L/kg for CH_4 per unit of DM and NDF, respectively. The disparity in CH_4 production between the studies containing extruded flaxseed may be due to varied crude fat concentration in the diet.

Martin et al. (2008) observed a decrease in CH_4 production. However, the crude fat as a percentage of DM was also increased (7.0 vs. 2.0% of DM); thus, it cannot be concluded that the observed effect was a result of increases in linolenic acid per se. In the current study, crude fat was similar but also could be considered low. It is possible that the total concentration of fat or, more specifically, total supply of linolenic acid was too low to induce the response hypothesized. It has been estimated that for each 1% increase in dietary fat, CH_4 production is reduced by 5.6% (g/kg of DMI; Knapp et al., 2014). This response is believed to be caused by 1 or a combination of several factors: (1) increased propionate concentration with altering microbial community, (2) providing an alternative hydrogen sink via biohydrogenation, and (3) providing less fermentable dietary substrates (Hales and Cole, 2017) or directly impeding fiber digestion. With the lack of response observed in the current experiment, we are unable to contribute additional knowledge on possible mechanisms, but future research should investigate manipulations of total fat concentration and fatty acid profile and associated effects on ruminal CH_4 production.

Nutrient Digestibility and Energy

Diets containing extruded flaxseed have found a great deal of variation in digestibility of nutrients. Martin et al. (2008) replaced extruded wheat and concentrate with extruded flaxseed fed to lactating cattle and observed a 5% reduction in DM and OM digestibility and a 25% reduction in NDF digestibility, whereas starch digestibility was not different. However, Martin et al. (2016) replaced corn grain and wheat bran with extruded flaxseed in diets fed to lactating dairy cattle and observed no difference in DM, OM, NDF, N, and starch digestibility in hay-based diets but observed a 25% reduction in NDF digestibility and a 3% increase in starch digestibility in corn silage-based diets. In the current study, no differences were observed in nutrient digestibility (Table 7). Hammond et al. (2015) replaced cracked wheat with extruded flaxseed and observed similar DM, OM, and CP digestibility. In a second study, these investigators observed a tendency for CP digestibility to increase with the inclusion of extruded flaxseed; this was not observed in this study. Polyunsaturated fatty acids are believed to be toxic to rumen microbes and may decrease NDF digestibility (Beauchemin et al., 2007). In addition, there is a positive association with degree of unsaturation of fatty acids and fatty acid digestibility; however, it potentially decreases ruminal fermentation with PUFA (NRC, 2001). With the potential negative effects of PUFA on fermentation, digestibility is a concern when feeding linolenic acid. However, in the current study, digestibility was not affected, which may have been the result of a lower dietary inclusion of fat. Many of the studies that demonstrated biological effects with the inclusion of flaxseed did so with diets containing 6 or 7% crude fat as a percentage of dietary DM. However, in the current study, crude fat was less than 5% of dietary DM. Although the concentration of linolenic acid increased in concentration with extruded flaxseed, the concentration may not have been great enough to

Table 6. Methane production and heat production of lactating Jersey cows in late lactation fed control (CON) or extruded byproduct containing flaxseed (EXF) diets

	Treatment			
Item	CON	EXF	SEM[1]	*P*-value
O_2 consumption, L/d	4,143	4,132	205.1	0.960
CO_2 production, L/d	4,346	4,357	200.6	0.959
CH_4 production, L/d	352	350	16.4	0.904
Respiratory quotient,[2] L/L	1.05	1.06	0.01	0.413
CH_4/DMI, L/kg per day	23.8	22.4	0.57	0.343
CH_4/milk produced, L/kg per day	22.7	19.8	0.95	0.300
CH_4/ECM, L/kg per day	16.5	14.5	0.68	0.303
CH_4/digestible DM, L/kg	35.0	33.5	1.92	0.531
CH_4/digestible NDF, L/kg	46.8	41.9	4.23	0.397
CH_4 energy, Mcal/d	3.33	3.31	0.15	0.904
Heat production,[3] Mcal/d	21.1	21.0	1.02	0.980
Heat production,[4] kcal/$MB^{0.75}$	213	217	7.79	0.685

[1]Lowest standard error of treatment means is listed.

[2]Calculated as CO_2 produced/O_2 consumed.

[3]Heat production calculated with Nienaber and Maddy's (1985) equation from O_2 consumption (L), CO_2 production (L), and CH_4 production (L); heat production (Mcal/d) = (16.18 × O_2 L + 5.02 × CO_2 L − 2.17 × CH_4 L)/4.183.

[4]Heat production per unit of metabolic BW.

Table 7. Apparent digestibility of nutrients and wet fecal output of lactating Jersey cows in late lactation fed control (CON) or extruded byproduct containing flaxseed (EXF) diets

Component, % unless noted	Treatment		SEM[1]	*P*-value
	CON	EXF		
DM	68.0	66.9	1.07	0.481
OM	70.2	69.6	0.95	0.629
CP	74.0	72.6	1.07	0.388
NDF	52.6	54.6	2.43	0.576
Starch	96.7	95.4	0.64	0.221
Fatty acid	79.1	78.6	2.76	0.903
16 Carbon	80.0	79.1	3.41	0.738
18 Carbon	79.9	82.0	2.91	0.626
Fecal output (wet), kg/d	28.2	29.3	2.09	0.605

[1]Lowest standard error of treatment means is listed.

elicit a large effect on the rumen environment. Based on the digestibility of nutrients listed in Table 7 and by assuming heats of combustion of 4.2 Mcal/kg for NDF and starch, 5.6 Mcal/kg for CP, and 9.4 Mcal/kg for fat, digestible energy can be calculated (NRC, 2001). Using this approach, the digestible energy of the control and EXF was observed to be 2.73 and 2.80 Mcal/kg, respectively. Because DMI and digestibility of nutrients were not affected by treatment, the modest differences in concentrations of digestible energy between treatments can be attributed to the small differences in the concentrations of fat. Energetic losses from CH_4 production are estimated to range from 2 to 12% (Johnson and Johnson, 1995). It has been suggested that a reduction of CH_4 by 25% could increase growth in beef cattle by 75 g of BW gain/d (Nkrumah et al., 2006) or milk production by approximately 1 L/d (Bruinenberg et al., 2002). In the current study, CH_4 energy can be calculated by multiplying the volume of CH_4 by 9.45 kcal/L (Moe and Tyrrell, 1979). Methane energy was very similar for CON and EXF treatments (3.33 and 3.31 Mcal/d, respectively) when calculated using total CH_4 production and is not likely to result in any differences in energy utilization.

CONCLUSIONS

The present study demonstrated that extruded flaxseed may be included in the diet as an alternative feed source without negative effects on lactation performance when fed to late-lactation Jersey cows. Inclusion of extruded flaxseed to increase linolenic acid did not affect DMI, milk yield, or milk components. Contrary to our hypothesis, CH_4 production was not decreased when the dietary concentration of linolenic acid was increased. Inclusion of extruded flaxseed up to 10% of DM had no negative effect on digestibility in late-lactation dairy cows.

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