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Methane mitigation in practically applicable dairy diets: Impact of diet composition and extruded linseed

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HIGHLIGHTS

- Effects of replacing grass silage with corn silage and of extruded linseed supplementation on enteric methane emissions in optimized diets for high-yielding dairy cows.
- Replacing grass with corn silage had no effect on production or methane emissions.
- Extruded linseed reduced methane production and yield in a corn-based diet.
- Adding extruded linseed to a grass-based diet had no impact on methane emissions.
- Extruded linseed as a methane mitigator depends on diet composition.
- Methane mitigation may fail when optimizing diets for high yielding dairy cows.

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ABSTRACT

Recognized strategies to mitigate methane (CH_4) emissions from dairy cows include increased levels of dietary starch or lipids. Most studies have tested extreme experimental diets that are rarely applied in practice, as farmers generally apply lower concentrations. In the present study we evaluated the effects of partial replacement of grass silage (GS) with corn silage (CS) and extruded linseed (EL) supplementation in optimized dairy cattle diets under conventional farming conditions. Three 15-week experiments were conducted in three periods: adaptation (3 weeks), pre-treatment (6 weeks), and treatment (6 weeks). A reference group receiving a control diet was compared to each treatment group. In EXP1, GS (65 % GS, 35 % CS) was partially replaced with CS (35 % GS, 65 % CS). In EXP2 and EXP3, EL (13.8 % of dietary DM, fat increased by 1.5 %) was added to a CS-based (EXP2) and to a GS-based diet (EXP3). No significant effect was observed on milk production and CH_4 emissions when replacing GS with CS. EL addition to the CS-based diet showed a trend toward a 9 % reduction in CH_4 production and yield ($p = 0.097$ and $p = 0.074$), while no significant effect of EL was observed for the GS-based diet. In summary, replacing GS with CS had no effect on production or CH_4 emissions, while adding EL to a CS-based diet showed potential for CH_4 mitigation. This study shows that recognized CH_4 mitigation strategies may not prove effective when diets are optimized based on practical guidelines for high-producing dairy cows.

1. Introduction

The agricultural sector undeniably contributes to climate change (IPCC, 2021). Ruminants are important contributors due to their production of enteric methane (CH_4) (Pereira et al., 2022), a potent greenhouse gas (GHG) with a global warming potential (GWP100) 28 times larger than carbon dioxide (CO_2) (IPCC, 2021). According to the Paris Agreement (UNFCCC, 2015), mitigation strategies must be implemented to limit global temperature rise to 1.5 °C. The diet of dairy cows plays a crucial role in the production of CH_4 emissions (Johnson

Abbreviations: CS, corn silage; DMI, dry matter intake; DIM, days in milk; EL, extruded linseed; FOM, fermentable organic matter; FPCM, fat and protein corrected milk; GS, grass silage; NEl, net energy lactation; MP, metabolizable protein; MUN, milk urea nitrogen; OEB, rumen degradable protein balance; OCC, open circuit chambers; VFA, volatile fatty acids.

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and Johnson, 1995). Modifications in forage composition or the addition of ingredients with mitigating properties may reduce these emissions (Beauchemin et al., 2008; Knapp et al., 2014; Olijhoek et al., 2025). Both the replacement of grass silage with corn silage (Brask et al., 2013; Doreau et al., 2014; van Gastelen et al., 2015) and the addition of extruded linseed (Benchaar et al., 2015; Hassanat and Benchaar, 2021; Martin et al., 2010) are proven mitigation strategies. Increasing the amount of starch stimulates propionate production and the use of H_2, resulting in less H_2 available for CH_4 formation (Moss et al., 2000; Pereira et al., 2022). Dietary lipids reduce enteric CH_4 production in several ways. (1) Lipids bypass ruminal fermentation; (2) polyunsaturated fatty acids have inhibitory effects on cellulolytic bacteria and protozoa, resulting in shifts towards more propionate production (Martin et al., 2010; Pereira et al., 2022); (3) ruminal biohydrogenation of unsaturated fatty acids acts as an alternative H_2 sink (only limited to 1–2 % of H_2 from CH_4 production) (Johnson and Johnson, 1995); and (4) lipids have a coating effect on structural carbohydrates (Ellis et al., 2008; Jenkins, 1993). Extruded linseed (EL), an excellent source of fat, is rich in α-linolenic acid, making it a promising source of dietary fat with a significant CH_4-mitigating potential (Martin et al., 2010; Mustafa et al., 2002; Petit, 2010).

In experimental settings, large differences between diets are often used to maximize the contrast in treatment effects. However, many studies compare rather extreme dietary compositions that tend not to align with common practice in commercial dairy farming. For example, studies investigating the effects of replacing GS with MS often use a complete substitution (Brask et al., 2013; Doreau et al., 2014), sometimes combined with high concentrate levels (≥50 %) (Doreau et al., 2014; van Gastelen et al., 2023). In contrast, typical dairy cow diets in Western Europe consist of approximately 60 % roughage, primarily a mixture of fiber-rich grass silage (GS) and starch-rich corn silage (CS) in varying proportions. A more practice-oriented approach was taken by Van Gastelen et al. (2015), who examined four forage-rich diets (80:20 forage-to-concentrate ratio) with increasing amounts of corn silage. In that study, all diets were supplemented with the same concentrate, regardless of forage composition. This is in contrast to commercial dairy farming, where forage mixtures are balanced with by-products, grains, compound feeds, and protein sources to better meet the energy and protein requirements to ensure optimal milk production of high-yielding cows.

Similarly, studies evaluating extruded linseed often include high levels of crude fat (e.g., 4 % in Benchaar et al., 2015; 6.47 % and 5.84 % in Hassanat and Benchaar, 2021; 4.1–7.6 % in Martin et al., 2010). Crude fat inclusion should generally be limited to 6 % to prevent adverse effects on production parameters (Patra, 2013). This potential risk, combined with the higher cost of lipid sources compared to other concentrate ingredients (Ariza et al., 2019), means that the inclusion of extruded linseed (EL) in commercially optimized dairy diets poses practical challenges. In practice, therefore, EL inclusion is often lower than in experimental studies (Ariza et al., 2019).

With these considerations in mind, the present study was designed to explore CH_4 mitigation strategies in accordance with practical feeding strategies where different ration compositions, supplemented with various additives, are used to meet the nutritional requirements of dairy cows. The aim of the study was to evaluate the impact of (1) partial replacement of GS with CS (EXP1) and (2) supplementation with a commercially viable amount of EL in both a CS-based diet (EXP2) and a GS-based diet (EXP3) on enteric CH_4 emissions. We hypothesized that CH_4 emissions would be reduced when a higher proportion of CS was included in the diet and that the inclusion of EL would lower enteric CH_4 production without negatively affecting production parameters in both diet types.

2. Materials and methods

All animal trials were carried out at the experimental dairy farm of ILVO (Flanders Research Institute for Agriculture, Fisheries and Food, Melle, Belgium). All experimental procedures were approved by the ILVO Animal Ethics Committee (EC2014–239).

2.1. Experimental design

All experiments lasted for 15 weeks and were divided into three periods: adaptation (3 weeks, without data collection), pre-treatment (6 weeks), and treatment (6 weeks). During the final week of the pre-treatment period and the treatment period, the cows were housed in open-circuit chambers (OCC; De Campeneere and Peiren (2014)). The first experiment (EXP1) included 12 animals, while the second (EXP2) and the third experiment (EXP3) included 10 animals. Fig. 1 shows the experimental design of EXP1, EXP2, and EXP3. In all experiments, animals were randomly divided into two homogenous groups: a reference group (REF; EXP1: $n = 6$, EXP2: $n = 2$ and EXP3: $n = 2$) and a treatment group (TRT; EXP1: $n = 6$; EXP2: $n = 8$ and EXP3: $n = 8$). The randomization was balanced for parity, days in milk, milk production (kg/day), milk protein (%), milk fat (%), dry matter intake (DMI, kg/day), and body weight (kg). Due to limited availability of the OCC ($n = 6$), animals of EXP1 were divided in two groups (A and B) containing REF animals (REF A: $n = 3$; and REF B: $n = 3$) and TRT animals (TRT A: $n = 3$; and TRT B: $n = 3$). In EXP 2 and EXP3, REF cows remained the same and stayed in the OCC for two consecutive measurement periods of 3 days. In EXP2, this measurement period was staggered by 1 week, while in EXP3 two measurement periods were performed consecutively. The TRT animals in EXP2 and EXP3 were also divided into two batches (TRT A: $n = 4$; and TRT B: $n = 4$). When data was analyzed, data collected in different REF and TRT batches within each experiment were considered as one group (REF and TRT). In EXP1, one reference cow was injured and left the trial, and emissions data of two cows (one REF and one TRT cow) were lost due to technical problems in one OCC. All data on these three cows were omitted. In EXP2, one TRT B cow left the trial due to illness. All data collected on this animal was removed.

2.2. Animals and housing

Each experiment was performed with a different set of lactating Holstein Friesian cows. At the start of the experiment, the 12 animals in EXP1 averaged (± SD) 97 ± 25 days in milk (DIM) and 31.5 ± 3.0 kg milk/day. EXP2 included 10 dairy cows, 190 ± 48 DIM, and a mean daily milk yield of 30.7 ± 4.5 kg. The 10 animals in EXP3 had average DIM of 276 ± 88 and a mean daily milk yield of 29.3 ± 2.7 kg. In EXP1, all cows were housed in a free stall with cubicles during each experimental period except for the last week. Animals of EXP2 and EXP3 were only housed in the free stall during the adaptation period. In both the pre-treatment and treatment period, cows of EXP2 and EXP3 were housed in a tie stall. During the last week of the pre-treatment and treatment period of each experiment, all animals were housed in the OCC for CH_4 measurement and sample collection. EXP1 consisted of 5 days of data collection in the OCC, whereas in EXP 2 and EXP3 consisted of only 3 days of data collection.

2.3. Diets and treatments

The ingredient composition of the control and treatment diet in all experiments is shown in

Table 1. In EXP1, the reference roughage mixture was a GS-based diet which consisted of 64 % pre-wilted GS, with 34 % corn silage and 1 % straw on dry matter basis and was offered as a partial mixed ration. The treatment diet of EXP1 was a CS-based roughage mixture, consisting of 36 % GS, 63 % CS, and 1 % straw on dry matter basis. The REF animals continued to receive the reference roughage mixture, while the TRT group received the treatment roughage mixture when the treatment period started (Table 1). In EXP2 and EXP3, the roughage mixture in the reference diet and treatment diet remained unchanged. In EXP 2, the

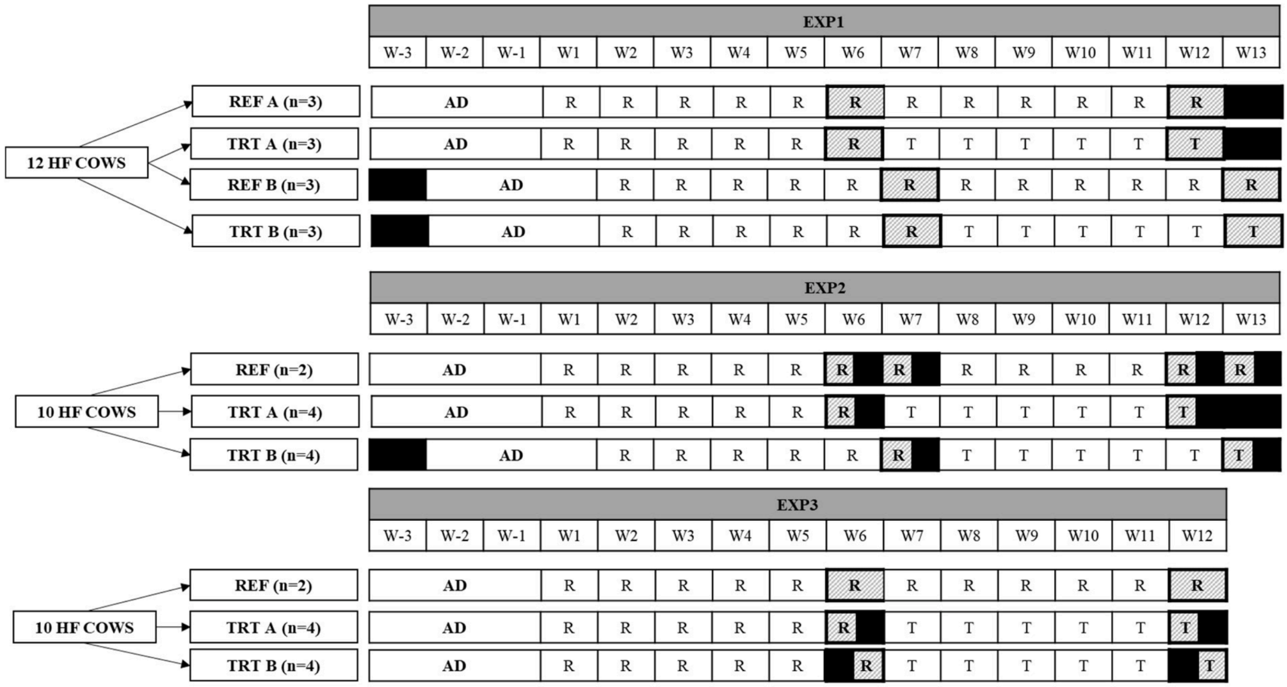

Fig. 1. Schematic overview of the experimental design of EXP1, EXP2 and EXP3. All experiments started with an adaptation period of 3 weeks (AD), followed by a pre-treatment period where animals received the reference diet (R), and a treatment period where TRT animals received the treatment diet (T) and REF animals continued to receive the reference diet. Both periods lasted for 6 weeks. The treatment period started upon completion of the pre-treatment period measurements in the OCC. Because the number of OCC was limiting ($n = 6$), the animals in EXP1 were divided into two batches (A and B) of 6 animals each which were further subdivided into REF animals (REF A: $n = 3$ and REF B: $n = 3$) and TRT animals (TRT A: $n = 3$ and TRT B: $n = 3$). In EXP2 and EXP3, animals were divided into one batch of REF animals ($n = 2$) and two batches of TRT (TRT A: $n = 3$ and TRT B: $n = 3$) animals were measured in separate weeks (shown in bold). In EXP2, REF animals ($n = 2$) were housed two consecutive weeks in the OCC, while TRT A and TRT B were housed in the OCC in separate weeks (shown in bold). In EXP3, REF animals were measured last week of each period, while TRT A animals were housed in the OCC in the first part of the last week and animals from TRT B were housed in the OCC in the second part of the last week (weeks shown in bold). Weeks shown with the dashed shading, are included in the statistical analysis. Black shaded cells are not included in the statistical analysis.

roughage mixture was based on CS and consisted of 65 % CS, 25 % pre-wilted GS, and 10 % pressed sugar beet pulp on dry matter basis. In EXP3, the roughage mixture was based on GS and consisted of 65 % GS, 25 % CS, and 10 % pressed sugar beet pulp on dry matter basis. Starting from the ad libitum intake of the roughage mixture, cows received concentrate supplementation (

Table 1) to fulfill 105 % of the individual requirements for net energy for lactation (NE_L, MJ/kg DM) and metabolizable protein and to fulfill a rumen degradable protein balance (OEB) of 200 g/day in EXP1 and 150 g/day in EXP2 and EXP3. The Dutch feed evaluation system was used to calculate the diets (Tamminga et al., 1994), which were formulated based on preliminary chemical analyses of the ingredients to ensure they were isoenergetic for all experiments and isonitrogenous for EXP1. To ensure sufficient starch provision in the GS diet in EXP1, a balanced concentrate rich in starch was used. In the CS diet, this starch-rich concentrate was exchanged for a balanced lower-starch concentrate. During the treatment periods of EXP2 and EXP3, the balanced concentrate of the treatment diet cows was partly replaced by an EL product (Linex 10/500, Arvesta NV, Belgium) consisting of 30 % linseed and other ingredients such as wheat (17 %), rapeseed meal (15 %), corn meal (8 %), and sunflower meal (11 %). The replacement was based on an equal NE_L provision.

2.4. Measurements and analysis

In all experiments, the roughage mixture was offered twice daily (8:30 am and 5:30 pm). Concentrates were offered four times a day in equal proportions (7:30 am, 11:00 am, 3:00 pm, and 5:30 pm) and DMI was measured, taking leftovers into account. All animals had free access to drinking water during the entire trial. Samples of all feed ingredients were taken once weekly, except for GS, which was sampled twice weekly. For chemical analysis, feed samples of CS and GS were pooled per period (pre-treatment period and treatment period) and all other ingredients were pooled over the whole trial. Silage samples were dried in a ventilated oven at 65 °C and all feed samples were ground using a 1 mm screen (Whiley, Rheotec). Next, samples were dried at 103 °C to obtain residual moisture content (EC, 1971a) and incineration at 550 °C to determine the crude ash content (ISO:5984, 2002). Kjeldahl was used to determine the crude protein ($N\times$ 6.25) content (ISO:5983–2, 2005). Petroleum ether was used to extract crude fat, for the concentrate ingredients, a hydrolyzing step with hydrochloric acid was performed beforehand (ISO: 6492, 1992). Crude fiber was obtained by the Ankom Fiber Analyser (AnkomTechnology, Macedon NY, USA) after boiling subsequently with sulphuric acid and sodium hydroxide (EC, 1992). The Ankom Fiber Analyser was used to determine the NDF, ADF and ADL. aNDFom was obtained using α-amylase and sodium sulfite and expressed on an ash-free basis (Van Soest et al., 1991). After drying and weighing, the residue was treated with sulphuric acid to determine ADLom. ADFom and ADLom were corrected for ash in the ADL residue (Van Soest et al., 1991). Starch was determined after autoclaving and hydrolysis with amyloglucosidase (NEN, 1974). Sugars were extracted with 40 % ethanol and analyzed according to the Luff–Schoorl method (EC, 1971b). The silage fermentation products and ammonia of the silages were determined on a water extract: lactic acid by an enzymatic method (Gawehn.; Noll, 1966), and acetic acid, propionic acid, butyric acid, and alcohols with gas chromatography (Jouany, 1981). The DM content and consequently the chemical composition of the silages were corrected for losses of volatile substances during drying ((Dulphy and Demarquilly, 1981). Additionally, the crude protein content was corrected for ammonia losses. (Dulphy and Demarquilly, 1981).

Table 1.
Mean composition of the diet on dry matter base (g/kg DM), and chemical composition (g/kg DM) of the total diets in both experimental periods (reference period (REF) and treatment period (TRT) of EXP1 (partially replacing GS with CS; REF: $n = 4$; TRT: $n = 5$), EXP2 (addition of EL to a CS diet; REF: $n = 2$; TRT: $n = 7$), and EXP3 (addition of EL to a GS diet; REF: $n = 2$; TRT: $n = 8$).

	EXP1				EXP2				EXP 3			
	PTP[1]		TP[2]		PTP		TP		PTP		TP	
	REF[3]	TRT[4]	REF	TRT	REF	TRT	REF	TRT	REF	TRT	REF	TRT
Ingredients of the diet (g/kg DM)												
Corn silage	254	245	240	430	470	447	464	458	178	176	178	178
Pre-wilted grass silage	444	428	475	247	183	174	188	186	452	448	468	470
Pressed beet pulp					81	77	81	80	85	84	82	82
Straw	7	7	7	10	8	8	8	8				
Soybean meal	46	42	44	54	35	35	35	35	4	10	4	10
Treated soybean meal[5]	17	18	16	18	8	16	7	17	18	11	17	11
Balanced concentrates	0	0	0	247	190	195	190	31				
Balanced concentrates starch	268	300	253	23					261	270	251	90
Rapeseed meal					19	42	19	43				
Extruded linseed product								138				160
Urea mix	0.4	0.2	0.4	6.7	6	4	6	5				
Chemical composition of total diet												
DM (g/kg)	464	472	496	487	514	530	515	521	484	488	492	494
Crude protein	164	164	163	163	143	150	144	157	146	144	147	154
Crude fat	29	29	30	28	30	30	28	43	29	29	30	46
Crude fiber	183	178	184	165	167	165	152	153	174	173	171	176
aNDFom	352	344	355	324	347	342	347	344	319	318	316	324
ADFom	205	200	213	193	192	190	194	195	186	184	187	194
Starch	189	195	189	252	233	225	220	200	163	166	159	122
Sugar	54	56	61	47	39	42	39	38	68	69	60	62
NE_L	4.27	4.30	4.19	4.30	4.39	4.40	4.36	4.39	4.53	4.54	4.49	4.51
MP (1994)[6]	92	94	91	91	90	98	89	95	92	91	91	87
OEB (1994)[6]	15	14	17	17	−2	−4	−2	6	0	−1	4	12
FOM	571	572	559	584	589	585	590	558	605	607	599	583

[1] PTP = pre-treatment period.
[2] TP = treatment period.
[3] REF = reference cows.
[4] TRT = treatment cows.
[5] Formaldehyde treated soybean meal, aNDFom = neutral detergent fiber (using α-amylase and sodium sulfite and expressed on an ash-free basis), ADFom = acid detergent fiber (expressed on ash-free basis) NE_L = Net energy lactation (MJ), MP = metabolizable protein, OEB = rumen degradable protein balance, FOM = fermentable organic matter.

Metabolizable protein (DVE) and rumen degradable protein balance (OEB) were calculated based on *in-sacco* measurements of rumen degradation and intestinal digestibility (Tamminga et al., 1994). The VEM of the feeds was predicted with regression equations (Boever et al., 1999) based on chemical composition and in vitro organic matter degradability using cellulase (De Boever et al., 1988). Cows were milked twice daily at 7:30 am and 5:30 pm. When housed in the OCC, milk samples were taken during four consecutive milkings and analyzed for milk fat, protein, lactose, and urea using Fourier-Transform infrared spectroscopy (FTIR) (Lactoscope Advanced, Delta instruments, Drachten, The Netherlands). Fat and protein corrected milk (FPCM) was calculated using the following formula: FPCM = Milk yield (MY) × [0.337 + (0.116 × milk fat %) + (0.06 × milk protein %) (Subnel et al., 1994). CH_4 and CO_2 emissions were continuously measured in the OCC (De Campeneere and Peiren, 2014) using an NDIR analyzer (MGA3500 Multi-Gas Analyser, ADC Gas Analysis Limited, UK). The air inlet and outlet were sampled every 16 min for 1 min and the ventilation rate was continuously measured.

2.5. Statistical analyses

For all experiments, only data collected when cows were housed in the OCC was used in this analysis. Data on DMI, MY, FPCM, %fat, % protein, % lactose, milk urea content (MUN) and CH_4 emissions were averaged for each cow and each measurement period (the last 5 or 3 days of the pre-treatment and treatment period). Statistical analysis was performed using the statistical software program R (EXP1: version 3.5.1 and for EXP2 and EXP3: version, 4.1.2, (www.r-project.org) and analyzed using a linear mixed model.

$$Y_{ijk} = G_i + P_j + (GP)_{ij} + C_k + \varepsilon_{ijk},$$

where Y_{ijk} is the dependent variable; G_i is the fixed effect of the i^{th} Group (i = REF, TRT), P_j is the fixed effect of the j^{th} period (j= pre-treatment period, treatment period); $(GP)_{ij}$ is the interaction effect of the i^{th} Group in the j^{th} period; C_k is the random effect of cow, and $\mathcal{E}_{ijk}$ is the residual error. The results are presented as least square means (LS means) per group and period. Differences were considered significant at $P \leq 0.05$ and a trend was considered as $0.05 < P \leq 0$ 0.1. A significant interaction effect was interpreted as a treatment effect, as it indicates a different evolution between the groups over the two treatment periods. The analyzed outcomes were assumed to be sufficiently normally distributed based on the graphical evaluation of the residuals of the models used (histogram and QQ-plot).

3. Results

3.1. Change from a GS-based diet to a CS-based diet

Changing from the GS-based diet to the CS-based diet in EXP1 did not significantly affect total DMI, MY, FPCM production, or milk composition (Table 2). Milk fat content showed a trend (p-value Group*-Period=0.10) toward evolving differently between REF cows and TRT cows because milk fat content for the cows that changed from the GS- to the CS-based diet decreased by 0.10 percent units. In contrast, milk fat content increased by 0.16 percent units for the reference cows during the same period. The replacement of GS with CS showed a significant effect (P-value Group*Period<0.05) on the intake of all nutrients except for the intake of OEB. When comparing the treatment period to the pre-

Table 2.
LS means of DMI, production of milk and fat-protein-corrected milk (FPCM), milk composition (fat, protein, lactose and MUN) in both experimental periods and for each group (reference cows (REF) and treated cows (TRT)) of EXP1 (partially replacing grass silage (GS) with corn silage (CS); REF: *n* = 4; TRT: *n* = 5), EXP2 (addition of extruded linseed (EL) to a CS diet; REF: *n* = 2; TRT: *n* = 7), and EXP3 (addition of EL to a GS diet; REF: *n* = 2; TRT: *n* = 8).

	PTP[1]		TP[2]			P-value		
Item	REF[3]	TRT[4]	REF	TRT	SEM[5]	Group	Period	Group*period
EXP1								
DMI (kg day^{-1})	20.6	20.3	21.8	21.4	1.07	0.846	<0.01	0.435
MY (kg day^{-1})	28.8	26.5	27.9	26.1	0.86	0.264	0.388	0.691
FPCM (kg day^{-1})	29.4	27.8	29.1	27.2	1.83	0.513	0.681	0.822
Milk composition								
Fat (%)	4.16	4.37	4.32	4.27	0.31	0.620	0.159	0.100
Protein (%)	3.41	3.41	3.43	3.46	0.17	0.989	0.665	0.755
Lactose (%)	4.75	4.55	4.64	4.52	0.09	0.076	0.0275	0.196
MUN (mg/L)	209	244	205	211	21.7	0.220	0.840	0.177
EXP2								
DMI (kg day^{-1})	22.4	23.5	20.9	21.7	1.4	0.491	<0.01	0.191
MP (kg day^{-1})	27.7	29.7	28.3	28.9	4.3	0.698	0.721	0.498
FPCM (kg day^{-1})	30.3	32.1	29.9	31.6	3.9	0.693	0.815	0.928
Milk composition								
Fat (%)	4.73	4.59	4.53	4.83	0.23	0.610	0.411	0.159
Protein (%)	3.59	3.56	3.31	3.44	0.25	0.918	0.054	0.334
Lactose (%)	4.67	4.65	4.64	4.56	0.06	0.730	0.368	0.285
MUN (mg/L)	198	199	170	203	23.2	0.967	<0.01	<0.01
EXP3								
DMI (kg day^{-1})	20.2	21.4	21.1	21.6	1.1	0.362	<0.001	<0.001
MP (kg day^{-1})	25.5	27.6	24.4	27.9	2.7	0.522	0.235	0.210
FPCM (kg day^{-1})	27.9	30.3	26.4	29.4	1.5	0.360	0.084	0.810
Milk composition								
Fat (%)	4.56	4.72	4.51	4.47	0.38	0.726	0.790	0.729
Protein (%)	3.76	3.74	3.66	3.42	0.23	0.939	0.151	<0.05
Lactose (%)	4.65	4.72	4.57	4.61	0.09	0.522	0.219	0.649
MUN (mg/L)	155	146	146	140	24	0.745	0.525	0.853

[1] PTP = pre-treatment period.
[2] TP = treatment period.
[3] REF: reference cows,[4] TRT: treated cows.
[5] Highest value of SEM is published in the table. DMI = Dry matter intake, MY = Milk production, FPCM = fat and protein corrected milk production, MUN = Milk urea nitrogen.

treatment period, the intake of ADL and sugar was lower in the TRT group while the intake of starch was higher. No significant differences among absolute enteric CH_4 production (g CH_4 day^{-1}), CH_4 yield (g CH4 kg DMI^{-1}), and CH_4 intensity (g CH_4 kg $FPCM^{-1}$) were observed between the GS-based diet and the CS-based diet (Table 3).

3.2. EL supplementation in a CS-based diet (EXP2)

Results of DMI, MY and milk composition are shown in Table 2. DMI was not significantly affected by EL supplementation. However, both REF and TRT showed a lower DMI during the treatment period

Table 3
. LS means of CH_4 emissions in both experimental periods and each group (reference cows (REF) and treated cows (TRT) in EXP1 (partially replacing grass silage (GS) with corn silage (CS); REF: *n* = 4; TRT: *n*= 5), EXP2 (addition of extruded linseed (EL) to a CS diet; REF: *n* = 2; TRT: *n*= 7), and EXP3 (addition of EL to a GS diet; REF: *n* = 2; TRT: *n*= 8).

	PTP[1]		TP[2]			P-value		
Item	REF[3]	TRT[4]	REF	TRT	SEM[5]	Group	Period	Group* period
EXP1								
g CH_4 day^{-1}	435	423	437	439	27	0.760	0.863	0.535
g CH_4 kg DMI^{-1}	21.1	20.9	20.0	20.5	0.8	0.790	0.114	0.441
g CH_4 kg MY^{-1}	15.3	16.0	15.9	16.8	1.2	0.648	0.181	0.779
g CH_4 kg $FPCM^{-1}$	15.1	15.3	15.2	16.3	1.3	0.874	0.703	0.257
EXP2								
g CH_4 day^{-1}	445	450	452	420	25.2	0.867	0.642	0.097
g CH_4 kg DMI^{-1}	19.8	19.2	21.6	19.4	1.2	0.675	<0.05	0.074
g CH_4 kg MY^{-1}	16.7	15.5	16.7	15.4	2.6	0.690	0.999	0.971
g CH_4 kg $FPCM^{-1}$	15.1	14.3	15.7	13.9	2.1	0.773	0.502	0.372
EXP3								
g CH_4 day^{-1}	493	499	460	442	23	0.844	0.280	0.515
g CH_4 kg DMI^{-1}	24.4	23.4	21.8	20.4	1.1	0.474	0.112	0.816
g CH_4 kg MY^{-1}	19.5	18.4	19.2	16.1	1.6	0.585	0.752	0.143
g CH_4 kg $FPCM^{-1}$	25.6	23.6	25.4	21.7	1.6	0.318	0.906	0.450

[1] PTP = pre-treatment period.
[2] TP = treatment period.
[3] REF: reference cows.
[4] TRT: treated cows.
[5] Highest value of SEM is published in the table. DMI = Dry matter intake, My = milk yield, FPCM = fat and protein corrected milk yield.

compared to the pre-treatment period ($P < 0.01$). This resulted in a lower ($P < 0.01$) intake of all nutrients, except for OEB in both groups. MY and milk composition were not significantly affected by EL addition. Milk urea nitrogen (MUN, mg l^{-1}) was reduced in the REF group but remained at a similar level in the TRT group (p-value Group*Period <0.01) when comparing the treatment period and the pre-treatment period. EL addition significantly influenced the intake of all nutrients except for crude fiber. When EL replaced the balanced concentrates, starch intake, DVE, and FOM decreased, while crude fat, crude protein intake, and OEB increased. NE_L intake decreased equally for both groups (REF and TRT) in the treatment period. Table 3 shows the effect on CH_4 emissions. Under EL supplementation to the CS diet in EXP2, absolute CH_4 production (g CH_4 day^{-1}) and CH_4 yield (g CH_4 kg DMI^{-1}), both show a trend ($P = 0.097$ and $P = 0.074$) toward a reduction of 9 %. When expressed per kg milk or kg FPCM, EL supplementation showed no significant effect nor trend toward significance.

3.3. EL supplementation in a GS-based diet (EXP3)

DMI of the REF cows increased while DMI of TRT cows remained stable over the two periods. Supplementation of EL significantly influenced the intake of all nutrients. On the GS diet, intake of starch, DVE, crude fiber, and FOM decreased, while intake of crude fat, crude protein, and OEB increased under EL supplementation. NE_L intake was reduced in the TRT group, while no notable changes were observed for the REF group when comparing the treatment period to the pre-treatment period. MY and milk composition (Table 2) were not significantly affected by the addition of EL, except for protein content (%). EL did not significantly affect the absolute CH_4 production, CH_4 yield, and CH_4 intensity (kg MY and kg FPCM production) (Table 3).

4. Discussion

4.1. The change from a GS-based diet to a CS-based diet

In the present study the effect of a partial substitution of GS with CS was evaluated for animal production and CH_4 emissions, with a focus on the implications for diets used on commercial dairy farms. Maintaining production is crucial in dairy farming, thus both diets were formulated to be isoenergetic and isonitrogenous. Protein demands, for example, must still be met when replacing GS with CS; this requires protein supplementation with products such as soybean meal. To fulfill net energy and more in particular glucogenic requirements (to support milk lactose output) diets rich in GS (> 40 % of total dry matter) are generally supplemented with additional sources of starch, such as cornmeal or wheat. Although no strict starch requirements have been published, commercially optimized diets for high-producing cows seldom have a starch content below 20 % of DM (Chase, 2007). In the current study, the GS-based diet had a starch content of 19 % of DM, compared to a starch content of 25 % in the CS diet. The GS diet was supplemented with a starch-rich balanced concentrate (197 g crude protein, 53 g crude fiber, 120 g aNDFom, 358 g starch, 129 g DVE per kg DM) and the CS diet was supplemented with a different balanced concentrate (180 g crude protein, 85 g crude fiber, 185 g aNDFom, 225 g starch, 122 g DVE per kg DM). In this trial, MY was not affected by the change from the GS to CS diet plus provision of their respective concentrates. Only a trend toward lower milk fat ($p = 0.10$) was found. According to Van Straalen and Tas (2010) half of the milk fat originates in acetic and butyric acid, which are produced mainly in the rumen and to a minor extent during hindgut fermentation. The cows on the CS-based diet were fed a diet with lower crude fiber, aNDFom, and sugar; one can therefore hypothesize that the absolute production of acetic, butyric acid and/or acetic acid to propionic acid ratio is lower, which may result in lower milk fat production (Bannink et al., 2006; Maxin et al., 2011). Several papers, when reviewing options for enteric CH_4 mitigation, state that (partial) replacement of GS with CS is a promising nutritional strategy to reduce CH_4 emissions (Beauchemin et al., 2008; Grainger and Beauchemin, 2011; Hristov et al., 2013). This is due to the starch content in corn silage, which favors rumen propionate at the expense of acetate production, resulting in lower CH_4 (Grainger and Beauchemin, 2011). Changes in milk fat content in our trial suggest reduced acetic acid production in the CS-based diet, but this was not reflected in CH_4 emissions. Van Gastelen et al. (2015) noted that a critical starch concentration is needed to reduce CH_4, as they found a quadratic decrease with increased corn silage. Unlike van Gastelen et al. (2015), the present trial replicated practical diets by adjusting the type and amount of concentrates based on the net energy and protein needs of the cow based on milk production and milk composition. Livingstone et al. (2015) also adjusted the contrasting diets to be isonitrogenous and to have a similar NDF and starch content. No differences in CH_4 production, yield, and intensity were found between their CS-based diet and their GS-based diet. In our experiment, NDF and starch intake differed significantly, but DMI and NEL intake were similar between diets, with no observed differences in CH_4 production, yield, or intensity (Table 2). Hammond et al. (2015) suggested that the source of NDF and starch can affect the rates of fermentation in the rumen and consequently affect CH_4 emissions, which can be related to inconsistent effects of the comparisons between GS and CS-based diets. Similar to van Gastelen et al. (2015), in both of our present experiments, feed intake was restricted (90 % of DMI in the tie stable) during the cows' stay in the OCC to avoid large individual differences in CH_4 and MY due to fluctuations in DMI. If ad libitum intake of roughages would have been possible in the OCC, the results could have been different because DMI is expected to be higher with corn silage (O'Mara et al., 1998; Grainger and Beauchemin, 2011; Hammond et al., 2015).

4.2. EL supplementation

In this study, as EL was added to different practical diets in two separate experiments, no direct comparisons between the two diets can be made. In general, it is known that dietary fats can have detrimental effects on DMI when supplemented in high amounts. However, these effects are heterogeneous and depend on the source, form, fatty acid composition, and amount of fat added to the total diet (Jenkins, 1993). A general threshold of 6 % fat content in the diet has been identified, below which no adverse effects on DMI are observed in lactating dairy cows (Livingstone et al., 2015; Martin et al., 2016) or growing dairy heifers (Hammond et al., 2015). Similarly, Meignan et al. (2017) showed that fat derived from EL could be added up to 2.9 % of the diet without negatively affecting DMI. The increased fat content of the TRT in both EXP2 and EXP3 (Table 1) remained well below both thresholds, thus no negative effects were expected. The interaction between Group and Period observed in EXP3 may not indicate a detrimental effect of EL on DMI, but might be related to the rather low DMI of the REF animals during the pre-treatment period. A meta-analysis showed an overall increase of 0.72 kg milk for each increase of 0.5 % in total fat content derived from EL (Meignan et al., 2017). In the present study, fat intake increased from 3.0 % up to 4.3 % and from 2.9 % up to 4.6 % in EXP2 and EXP3 respectively, albeit without a significant increase in milk yield. Both diets were formulated to be isoenergetic with decreasing starch content and increasing fat content, resulting in a shift from glucogenic to lipogenic energy. In isoenergetic diets, shifts such as these mainly affect milk composition rather than milk yield (van Knegsel et al., 2007). A decrease in protein content was only observed when EL was added to the GS diet. One proposed explanation for the reduction in protein content following dietary fat supplementation is a decreased availability of glucose precursors, as suggested by Wu and Huber (1994). This effect was probably only observed in the GS diet due to its inherently lower absolute starch amount and the greater reduction in starch after EL supplementation, which together may have limited the supply of glucose precursors required for milk protein synthesis. However, total protein yield (g day^{-1}) was not significantly influenced by the addition of

EL. Only Period showed an effect (P< 0.05, data not presented), with a decrease in both groups (REF and TRT) in the treatment period compared to the pre-treatment period. A dilution effect of the protein content can therefore be assumed (Meignan et al., 2017). Neither EL experiment showed an effect on milk fat content under EL addition. Literature shows heterogeneous results. Our results are in agreement with some authors (Livingstone et al., 2015; Martin et al., 2016), while others found a contrasting decrease in milk fat content (Fuentes et al., 2008; Hurtaud et al., 2010). Given the knowledge that polyunsaturated fatty acids are precursors to induce milk fat depression, diets in the present study contained probably enough fiber to counteract this negative side effect (Bauman and Griinari, 2003). Due to a lower DMI of both the REF and the TRT cows fed on the CS diet in the treatment period, the OEB decreased. In contrast, the addition of EL led to an increased OEB which in turn increased the MUN. This is due to a positive OEB, which increases the ammonia production in the rumen. The excess is absorbed through the rumen wall and is converted in the liver to urea that in turn perfuses all bodily fluids, including milk (Tamminga, 2006).

Unlike carbohydrates, lipids are not fermented in the rumen (Jenkins, 1993). Substituting carbohydrates by lipids is thus expected to reduce CH_4 production. However, the present study only revealed a trend toward reduced CH_4 production and yield when the CS-based diet was supplemented with EL. Hammond et al. (2015) found no significant reduction in CH_4 production and CH_4 yield when EL was added to CS-based and GS-based diets in two experiments with dairy heifers. In their experiments, the oil content increased from 3.61 % to 4.93 % and from 4.15 % to 5.46 % for CS and GS diets in the EXP1 and from 3.56 % to 4.48 % and from 3.68 % to 5.53 % for CS and GS diets in the EXP2. Livingstone et al. (2015) found the same lack of an effect when adding EL to a CS and a GS diet, resulting in an increase in total FA content (%) from 2.19 % to 3.13 % and from 2.18 % to 2.86 % for the CS and GS diet, respectively. Both studies assumed that a too-low increase in fat content explained the lack of effect on CH_4 emissions (Hammond et al., 2015; Livingstone et al., 2015). In contrast, the dose-response study of Martin et al. (2016) showed in both a hay-based and a CS-based diet a reduction in CH_4 production (−15 % and −6 %) and CH_4 yield (−7 % and −4 %) starting at the lowest dose of EL (from 2.3 % to 4.1 % increase in total FA in the diet). In our study, the fat content increased from 3.0 % to 4.3 % and from 2.9 % to 4.6 % in EXP2 and EXP3, respectively, which according to Martin et al. (2016) should have been sufficient to reduce the CH_4 production. When EL was added to the CS diet, CH_4 production and CH_4 yield tended toward a reduction of the same magnitude (9 %). This indicates that CH_4 emissions are reduced by other mechanisms than a lower DMI. EL supplementation led to a reduction in FOM of the diet, as a part of the carbohydrates (mainly starch) was replaced by fat. In an attempt to balance the net energy of the REF and TRT diet in EXP2 and 3, the FOM difference between the reference and the treatment diet was considerably smaller in EXP 3 (−2.7 % change in FOM content between TRT vs REF) than in EXP2 (−5.4 % change in FOM content between TRT vs REF). This could partly explain the presence and absence of a trend toward an effect of EL in EXP2 and EXP3, respectively. In our experiments, the supplementation of the same amount of EL in the CS-based diet only led to a trend toward a significant decrease in CH_4 production and yield. Similar results were found by Chung et al. (2011) where ground linseed led to a reduction (−36 % and −33 %) in CH_4 production (−36 %) and CH_4 yield (−33 %) in a barley silage-based diet. When the same amount was added to a hay-based diet, no reduction was found as the depressive effects (e.g., lower protozoal number, impaired ruminal fermentation, digestibility) were less prominent. When FA derived from linseed oil were added either to a CS diet or a red clover silage-based diet, both resulted in a reduction in enteric CH_4 emissions (−9 % and −26 %, respectively). However, the addition of FA led to reductions in DMI, digestibility, protozoal count, acetate to propionate ratio, and milk production but only in the CS diet (Benchaar et al., 2015). Overall, a larger CH_4-suppressing effect of FA can be observed in diets that are higher in starch content (Patra, 2013). Numerically, the intake of starch in EXP2 was higher (233 and 225 g kg DM^{-1} in the pre-treatment period and 220 and 200 g kg DM^{-1} in the treatment period for the REF and TRT animals, respectively) compared to the intake in EXP3 (163 and 166 kg day^{-1} in the pre-treatment period and 159 and 122 kg day^{-1} in the treatment period for the REF and TRT animals, respectively). However, in the present study statistical comparisons cannot be made between these experiments. More research is needed to gain more insight into this interaction.

5. Conclusions

The present study revealed no effect of partial replacement of GS with CS in accordance with optimized diets used in agricultural practice on enteric CH_4 emissions, milk production and milk protein content. Only milk fat content was decreased because of the increased levels of starch and decreased levels of crude fiber. Use of an EL product to increase the dietary fat amount to 4.2 % resulted in a trend toward a reduction in CH_4 yield of 9 % in the CS diet but not in the GS diet. The addition of EL had no effect on animal production in either diet. The results of this study suggest an interactive effect between the mitigating potential of EL and the composition of the basal diet. However, further experiments with a larger number of animals are needed to confirm this, as well as more mechanistic research to better understand the interaction with the diet composition. To conclude, mitigation strategies (e.g., increasing dietary starch and supplementing with sources of unsaturated fatty acids such as extruded linseed) may fail to meet their theoretical potential when diets are optimized according to practical guidelines for formulating rations for high-producing dairy cows.

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used ChatGPT in the writing process to improve readability. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

CRediT authorship contribution statement

J. Van Mullem: Writing – review & editing, Writing – original draft. **D. Van Wesemael:** Writing – original draft, Project administration, Investigation, Formal analysis. **B. Ampe:** Writing – review & editing, Formal analysis. **V. Fievez:** Writing – review & editing, Supervision. **L. Vandaele:** Writing – review & editing, Supervision, Methodology, Conceptualization. **N. Peiren:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

This research was performed by J. Van Mullem (ILVO/Ghent university), Dr. D. Van Wesemael (ILVO), Dr. B. Ampe (ILVO), Prof. Dr. V. Fievez (Ghent university), Dr. L. Vandaele (ILVO), and Dr. N. Peiren (ILVO). With the submission of this manuscript, the authors affirm that the content of this manuscript has not been published elsewhere, accepted for publication elsewhere or under editorial review for publication elsewhere; and that the representatives of the Institutes ILVO and Ghent University are fully aware of this submission. We have no conflicts of interest to disclose.

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