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# Dietary linseed and starch supplementation decreases methane production of fattening bulls

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ABSTRACT

The objective was to determine $CH_4$ production from bulls fed a feedlot diet rich in either fibre (F) or starch and lipid (SL) over the fattening period. Fifty six Charolais bulls (259 ± 9.4 d of age and 339 ± 8.2 kg live weight (LW)) were allocated randomly to one of two diets and blocked with 4 replicate pens/diet based on LW and age, and fattened for up to 18 months. Both treatments included barley straw with the appropriate concentrate mixture rich in fibre or starch and fat. The concentrate mixture and barley straw were available *ad libitum*, and the intake ratio (870:130; dry matter (DM) basis) for the concentrate mixture and barley straw was similar for both diets. Methane production was determined for each bull for 5 d using the sulfur hexafluoride tracer gas method at the beginning (24 d on diet ± 3.4), middle (120 d ± 8.2), and end (228 d ± 11.1) of the fattening period. Feed intake was measured daily and bulls were weighed every 15 d. Ruminal fluid samples were collected on the last day of each $CH_4$ measurement period by rumenocentesis and measured for pH and concentrations of volatile fatty acids (VFA). Bulls fed SL had lower DM, organic matter and gross energy intake ($P<0.05$) than bulls fed F. Bulls fed SL had lower $CH_4$ production (L/d and L/kg LW gain) than bulls fed F (−20% and −24%, respectively, $P<0.01$). Bulls fed SL had higher $CH_4$ (g/kg DM intake) than bulls fed F (+20%, $P<0.05$) at the end of fattening. Bulls fed SL had lower ruminal VFA and a lower acetate relative proportion than bulls fed F (−13% and −11%, $P<0.001$, respectively), but diet had no effect on ruminal pH. Body LW gain was 1.49 *versus* 1.58 kg/d ($P=0.05$) for bulls fed F and SL, respectively, during the first 200 d of fattening. There was no relationship between $CH_4$ production as L/d or L/kg LW gain and residual feed intake. Supplementation with extruded linseed lipids combined with starch in feedlot diet decreased enteric $CH_4$ emissions, as L/d, L/kg LW and L/kg LW gain, from fattening bulls, mainly due to lower DM intake, with reduced $CH_4$ emissions as L/d, L/kg LW and L/kg LW gain persisting throughout the fattening period.

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## 1. Introduction

Production of greenhouse gases (GHG) from livestock and their impact on climate change is a worldwide concern (Steinfeld et al., 2006). It has been reported that enteric $CH_4$ is one of the most important anthropogenic GHG emitted at the farm level in ruminant production systems. It is the main contributor to livestock GHG ranging from 56 to 65% in New Zealand dairy farms and from 48 to 65% in bovine milk production systems (Basset-Mens et al., 2009). Similarly, in meat production systems in France, enteric $CH_4$ contributed 58–66% of GHG emitted on farm (Veysset et al., 2010). Moreover, energy loss from animals due to $CH_4$ production ranges is 2–12% of gross energy (GE) intake in mature cattle (Johnson and Johnson, 1995).

Several factors contribute to variations in $CH_4$ production among animals of different breeds or among animals of the same breed, with differences in feed intake and proportion of concentrate in the diet being frequent contributors. Recently, several authors have reviewed a variety of strategies to mitigate enteric $CH_4$ production through biotechnologies such as use of feed additives as well as nutritional and genetic means (Beauchemin et al., 2009; Martin et al., 2010a). Among nutritional strategies, high concentrate and lipid supplementation is frequently most effective at lowering $CH_4$ (L/d or per unit dry matter (DM) intake). Sauvant and Giger-Reverdin (2009) observed a decrease in $CH_4$ production/unit GE intake when the proportion of concentrate was >40% of DM intake, and was only 2–3% of GE intake of diets containing >80% of concentrate. Lipid supplementation of diets fed to lactating dairy cows decreased production of $CH_4$ by 5% for each 10 g/kg added fat in the diet (Eugène et al., 2008) and $CH_4$ production intensity (g/kg DM intake) was reduced by 5.6% for each 10 g/kg added fat in the diets of small and large ruminants (Beauchemin et al., 2009). Among available fat sources, linseed is one of the most efficient for $CH_4$ mitigation (Martin et al., 2010a), and its practical interest also comes from its potential to increase the omega 3 fatty acid content of beef meat (Doreau et al., 2011). Combined effects of a moderate amount of supplementary fat with a high concentrate diet in fattening bulls on $CH_4$ production, feed intake and animal growth is not known.

Residual feed intake (RFI) in beef cattle, which is the difference between actual feed intake and expected feed intake to meet net energy requirements for maintenance of body weight (LW) and growth, has been shown to be moderately heritable (Arthur et al., 2001) and could be used to select low and high RFI divergent selection lines (*i.e.*, more and less efficient animals). Moreover, the few studies relating $CH_4$ production to RFI indicate that cattle producing less $CH_4$ also have low RFI (Nkrumah et al., 2006; Hegarty et al., 2007). However, the relationship between $CH_4$ production and RFI of bulls fed both lipid and starch supplemented diets is unknown.

Our aims were to compare effects of a diet rich in starch and lipids to a diet rich in fibre on $CH_4$ production in bulls fed high concentrate diets, to determine if differences between diets persisted during the fattening period and to determine the relationship between RFI and $CH_4$ production of bulls fed these diets.

## 2. Materials and methods

### 2.1. Animals, experimental design and dietary treatments

Fifty-six Charolais bulls at $259 \pm 9.4$ d of age and a live weight (LW) of $339 \pm 8.2$ kg were blocked by LW and age and randomly assigned to one of two dietary treatments and housed in 8 pens with 7 bulls each. Bulls were fattened for a minimum of 228 d prior to slaughter. Measurements of $CH_4$ production and rumen fermentation parameters of each bull were repeated 3 times at the beginning (Period 1: $283 \pm 9.9$ d of age), middle (Period 2: $380 \pm 12.5$ d) and end (Period 3: $488 \pm 13.4$ d) of the fattening period. Measurements of feed intake were recorded every day and LW every 15 d. Experimental procedures were conducted in accordance with the French Ministry of Agriculture guidelines for the use of experimental animals and animal welfare (www2.vet-lyon.fr/ens/expa/acc_regl.html).

Dietary treatments consisted of a diet rich in fibre (F) and a diet rich in starch and supplemented with lipids (SL). Both diets were composed of a concentrate mixture and barley straw fed *ad libitum*. Concentrate in the F diet on a fresh basis consisted mainly of cereal by-products, dehydrated lucerne and dehydrated beet pulp. Concentrate in the SL diet consisted of cereals and an extruded mixture (120 g/kg DM of concentrate) containing (DM basis) linseed (500 g/kg), wheat bran (300 g/kg) and sunflower meal (200 g/kg), to achieve a theoretical level of 12 g/kg DM of concentrate as omega-3 fatty acids from linseed (Valorex, Combourtillé, France). Ingredients and chemical composition of the F and SL diets are in Table 1. Diets were formulated to meet net energy and protein (*i.e.*, absorbable protein delivered to the intestinal absorptive site) requirements for maintenance and body weight gain (1.5 and 1.8 kg/d, for F and SL diet, respectively) of bulls, according to the French national institute for agronomical research recommendations (Garcia et al., 2007). A commercial mineral/vitamin premix was added to the concentrates at the level of 8 and 5 g/kg of concentrate DM for F and SL diets, respectively.

During the experiment, bulls were housed in pens (7 $m^2$/bull) bedded with barley straw. Bulls were individually fed the concentrate using an automatic feeding system (Calan system, Drafhandel Decuyper, Brussels, Belgium). Both concentrates were produced as pellets (Jacques Coeur, Joigny, France) and the straw was not chopped. Concentrate was fed once daily at 08:00 h for *ad libitum* intake. Each bull was equipped with an electronic transponder (Dairy gate, EFEI, Villeroy, France) around its neck which allowed opening of its own feeder, which was filled with concentrate with the amount provided being adjusted every two days to ensure 100 g/kg refusals. Straw was available continuously in a rack that was in each pen. Only mean intake of straw from the rack was measured for all bulls in a pen. It is possible that the bulls may have eaten some

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**Table 1**
Ingredients and chemical composition of the fibre (F) and starch and lipid diets (SL) fed to bulls.

| Ingredient g/kg (on a as fed basis) | Forage diet | Starch–lipid diet |
|---|---|---|
| Dehydrated lucerne, 170 g.kg CP | 224 | 0 |
| Bran, wheat | 219 | 0 |
| Bran, maize | 184 | 30 |
| Beet pulp, dehydrated | 212 | 60 |
| Rapeseed meal | 35 | 214 |
| Maize grain | 0 | 280 |
| Barley grain | 25 | 98 |
| Oat grain | 0 | 86 |
| Extruded mixture (500 g.kg linseed)[a] | 0 | 120 |
| Soyabean meal, 480 g/kg CP | 0 | 20 |
| Palm kernel meal | 40 | 0 |
| Molasses, liquid | 30 | 70 |
| Urea | 0 | 4 |
| Mineral–vitamin premix[b] | 8 | 5 |
| Magnesium oxide | 10 | 0 |
| Calcium carbonate | 10.3 | 10.3 |
| Sodium bicarbonate | 3.6 | 3 |

| Chemical composition[c] | F concentrate | SL concentrate | Barley straw |
|---|---|---|---|
| Dry matter (g/kg) | 902 | 885 | 913 |
| Organic matter (g/kg DM) | 800 | 824 | 852 |
| aNeutral detergent fibre (g/kg DM)[d] | 379 | 196 | 601 |
| Acid detergent fibre (g/kg DM) | 206 | 104 | 383 |
| Crude protein (g/kg DM) | 159 | 200 | 41 |
| Ether extract (g/kg DM) | 28 | 47 | – |
| Starch (g/kg DM) | 75 | 337 | 31 |
| Gross energy (MJ/kg DM) | 18.9 | 19.7 | 19.3 |

[a] The extruded mixture of SL diet contained linseed (500 g/kg DM), wheat bran (300 g/kg DM) and sunflower meal (200 g/kg DM).
[b] Mineral–vitamin premix (g/kg): Ca, 86; Mg, 10; Cu, 8; Zn, 40; Mn, 100; I, 0.48; Co, 0.08; Se, 0.08; vitamin A, 320,000 IU; vitamin D3, 160,000 IU; vitamin E, 800 IU; vitamin B1, 400 IU.
[c] Determined using samples pooled by diet within each experimental period.
[d] Neutral detergent fibre is aNDF, assayed with heat stable amylase and expressed inclusive of residual ash, and Acid detergent fibre is ADF.

straw used as bedding, but this amount was not measured. Fresh water was available at all times in each pen. The 56 bulls were gradually adapted to the diet treatment over 21 d.

### 2.2. Experimental measurements and sampling

Measurement of enteric $CH_4$ production on 5 consecutive days was repeated 3 times at the beginning, middle and end of the fattening period when the animals were on their respective diets for 24 d ± 3.4, 120 d ± 8.2, and 228 d ± 11.1, respectively. The sulfur hexafluoride ($SF_6$) tracer technique (Johnson et al., 1994) was used as described by Martin et al. (2008). Briefly, brass permeation tubes (12.5 mm × 40 mm i.d.), weighing about 32 g, were charged with about 600 mg of $SF_6$ (Air Liquide, Mitry-Mory, France) while in liquid N. Subsequently, they were immersed in a water bath at 39 °C and weighed twice weekly for an 8 week period to establish $SF_6$ permeation rates. Permeation rates of $SF_6$ from the tubes did not differ between F and SL diets and were 1052 ± 130 ng/min and 918 ± 203 ng/min, respectively. A permeation tube was introduced orally into the rumen of each bull 1 week before the first gas sampling. Representative breath samples from each bull were collected into pre-evacuated (−91.2 kPa) polyvinyl chloride collection devices (~2.5 L) by means of capillary and Teflon tubing fitted to a halter (Martin et al., 2008). The pre-evacuated collection devices were placed in a bag fixed on the back of the bulls and changed at 07:00 h before the morning feeding every 24 h. The devices containing air samples were transported to the laboratory and over pressurized with $N_2$ gas to 142.0 kPa before $SF_6$, $CH_4$ and $CO_2$ analyses. Background concentrations of gases were measured daily in ambient air samples collected in a device placed in the barn. Gas was collected from 3, 3 and 2 pens of bulls in week 1, 2 and 3, respectively, of the 3 gas collection periods. The same pens of bulls were sampled in the same week of each measurement period for the 3 periods.

During gas collection, feed intake was measured on 5 consecutive days and the amount of concentrate offered was adjusted daily. Otherwise, feed intake was measured with the amount of concentrate offered being adjusted every 2 d and the bulls being weighed every 15 d. LW gains were calculated from day 1 to 70, 70 to 140 and 140 to 200.

Rumen fluid samples were collected once before the morning feeding on the last day of each period of gas collection. A 30 ml sample of rumen liquid was collected by rumenocentesis (Kleen et al., 2004). Rumen liquid pH was immediately measured using a digital pH meter (CG840, electrode Ag/AgCl, Schott Geräte, Hofheim, Germany). The liquid was then filtered (250 μm nylon filter), and an aliquot (0.8 ml) of the filtrate was mixed with 0.5 ml of 0.4% (w/v) crotonic acid and 2% (v/v) metaphosphoric acid in 0.5 M HCl and centrifuged at 16500 × *g* at 4 °C for 10 min. Supernatant was stored at −20 °C for subsequent VFA analysis.

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### 2.3. Laboratory analyses and calculations

#### 2.3.1. Intake

Two samples of each feed ingredient were collected every week during gas collection and composited for each experimental period. The DM content was determined on the first set of samples at 103 °C for 24 h (AOAC, 1990; #934.01). The second set of samples were ground to pass a 0.8 mm screen and analysed for ash by combustion at 550 °C for 6 h, N by a Kjeldahl procedure (AOAC, 1990; #976.05); aNDF (assayed with a heat stable amylase and expressed inclusive of residual ash; Van Soest et al., 1991), acid detergent fibre (ADF) by Van Soest et al. (1991), starch by a polarimetric method (AFNOR, 1985), ether extract (EE) according to AOAC (1990; #920.39) and GE using an adiabatic bomb calorimeter (Gallenkamp Autobomb; Loughborough, Leics, UK).

#### 2.3.2. Methane production

Concentrations of $SF_6$ and $CH_4$ in respired and ambient gas were determined by gas chromatography using a chromatograph (Varian-Chrompack, CP-9003, Les Ulis, France) fitted with electron capture detector for $SF_6$ and a second chromatograph (Perkin Elmer instruments, Autosystem XL, Courtaboeuf, France) fitted with a flame ionisation detector for $CH_4$. Samples were run on the gas chromatograph using a 0.5 nm molecular sieve column maintained at 50 °C for $SF_6$ and a Porapak N 80–100 mesh column maintained at 40 °C for $CH_4$. The flow rate of the carrier gas was 30 ml/min for $N_2$ and 40 ml/min for He for $SF_6$ and $CH_4$, respectively. The chromatographs were calibrated to obtain the response factor of detection using standard gases (Air Liquide, Mitry-Mory, France) for $SF_6$ (195 ppt) and $CH_4$ (100 ppm). Daily $CH_4$ production from each bull was calculated according to Johnson et al. (1994), using the known permeation rate of $SF_6$ and the concentrations, corrected for background, of $SF_6$ and $CH_4$ in the respired samples: $CH_4$ (L/d) = $SF_6$ permeation rate (L/d) × [$CH_4$]/[$SF_6$].

#### 2.3.3. Ruminal VFA

Volatile fatty acids were determined in ruminal fluid filtrate using crotonic acid as the internal standard and a CP 9002 Gas Chromatograph (Chrompack, Middelburg, Germany) as described by Morgavi et al. (2003).

#### 2.3.4. Residual feed intake

Residual feed intake (RFI) was calculated as the difference between actual and predicted feed intake, where the latter was estimated from the regression of daily feed intake on average metabolic LW (*i.e.*, $LW^{0.75}$) and LW gain over the first 200 d of the experiment described by Arthur et al. (2001). The residuals were then used as the measure of $RFI_{200\,d}$. Although feed intake data was measured during the entire experiment, intake data beyond 200 d were not included in order to be consistent with LW gain measurement, which was calculated over 200 d. Initial LW (*i.e.*, initial $LW_{200\,d}$) and final LW (*i.e.*, final $LW_{200\,d}$) over the first 200 d were determined, and average LW (*i.e.*, $LW_{200\,d}$) was calculated as:

$$LW_{200\,d} = \frac{\text{initial}\ \ LW_{200\,d} + \text{final}\ \ LW_{200\,d}}{2}$$

Liveweight gain (*i.e.*, LW $gain_{200d}$) was calculated as:

$$LW\ \ gain_{200\,d} = \frac{\text{final}\ \ LW_{200\,d} - \text{initial}\ \ LW_{200\,d}}{200}$$

### 2.4. Statistical analysis

Analysis of variance was completed on DM intake, LW gain, $CH_4$, VFA and pH data using the MIXED procedure of SAS (2000). One bull per diet was removed from the experiment due to health related complications during period 2, and 2/diet in period 3. The statistical model included diet, period, pen within diet and their interactions as fixed effects and bull within pen as a random effect. Period was treated as a repeated measurement and an unstructured covariance structure was chosen, based on the lowest Akaïke's criteria. For DM intake, as forage intake was pen based, the statistical model included diet, period and their interactions as fixed effects and pen as a random effect. Multiple comparisons among LS means used the PDIFF and Adjust = Tukey option of SAS (2000). Regression analyses were also completed between $CH_4$ production (L/d) and RFI using regression procedure of SAS (2000). Significance was declared if $P<0.05$ and a tendency to significance if $0.05<P\leq0.10$.

## 3. Results

### 3.1. Intake and growth

The concentrate:straw ratio for ingested feeds averaged 870:130 on a DM basis for both diets. During $CH_4$ sampling, bulls fed SL had lower DM, OM and GE intake ($P<0.001$) than bulls fed F. The extent of the difference was higher at the middle and end of fattening than at the beginning of fattening (Table 2). Bulls fed SL had a lower intake expressed as DM intake/LW as

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**Table 2**
Intake of bulls fed either a fibre diet (F) or a diet rich in starch and lipid (SL).

| | P1[a] | | P2 | | P3 | | SEM | P | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | F[b] | SL | F | SL | F | SL | | Diet | Period | Diet*period |
| DM intake (kg/d) | 8.2 a | 7.1 b | 11.3 a | 8.8 b | 10.0 a | 7.9 b | 0.28 | <0.001 | <0.001 | 0.009 |
| OM intake (kg/d) | 7.5 a | 6.6 b | 9.1 a | 7.4 b | 8.2 a | 6.6 b | 0.24 | 0.002 | <0.001 | 0.05 |
| Gross energy intake (MJ/d) | 168 a | 152 b | 216 a | 165 b | 183a | 154 b | 5.4 | 0.001 | <0.001 | <0.001 |
| DM intake/LW (kg/100 kg) | 2.2 a | 1.9 b | 2.1 a | 1.7 b | 1.5 a | 1.1 b | 0.05 | <0.001 | <0.001 | 0.35 |

Different letters (a, b) within the same period indicate differences between the diets P<0.05.
[a] Period 1–3 (P1–P3): the beginning (24 d), middle (120 d), and end (228 d) of the fattening period of bulls.
[b] F: diet composed of 0.87 concentrate rich in fibre and 0.13 straw, SL: diet composed of 0.87 concentrate rich in starch and fat (from linseed) and 0.13 straw.

**Table 3**
Intake and liveweight (LW) gain during the first 200 d of fattening of bulls fed a fibre (F) or a diet rich in starch and lipid (SL).

| | F[a] | SL | SEM | P |
|---|---|---|---|---|
| DM intake$_{200\,d}$ (kg/d)[b] | 10.3 | 8.7 | 0.17 | <0.001 |
| Initial LW$_{200\,d}$ (kg) | 352.8 | 341.2 | 5.40 | 0.14 |
| Final LW$_{200\,d}$ (kg) | 660 | 660 | 13.4 | 0.9 |
| LW gain $_{200\,d}$ (kg/d) | 1.49 | 1.58 | 0.028 | 0.05 |
| [c]DM intake/LW gain (kg/kg) | 7.0 | 5.6 | 0.15 | <0.001 |

[a] F: diet composed of 0.87 concentrate rich in fibre and 0.13 straw, SL: diet composed of 0.87 concentrate rich in starch and fat (from linseed) and 0.13 straw.
[b] DM intake and LW gain were averaged and calculated during the first 200 d.
[c] Feed conversion ratio expressed either as DM intake/LW gain or net energy for gain intake/LW gain.

kg/100 kg (P<0.001). Throughout the first 200 d, bulls fed SL had lower DM intake and DM intake/LW gain as kg/kg (P<0.0001) than bulls fed F (Table 3).

### 3.2. Methane production

Bulls fed SL had lower (P<0.001) $CH_4$ production as L/d than bulls fed F (Table 4). Bulls fed SL had higher $CH_4$ production as L/DM intake than bulls fed F (+20%; P<0.05) at the end of fattening, whereas there were no differences at the beginning and middle of fattening. There was no difference between diets for $CH_4$ production expressed as L/OM intake or as % of GE intake. Bulls fed SL diet had lower (P<0.0001) $CH_4$ production as L/kg LW and L/kg LW gain than bulls fed F (Table 4). The extent of the difference in $CH_4$ production as L/kg LW gain between bulls fed L and SL was higher (P=0.05) at the end of fattening than at other sampling points. Throughout the first 200 d, bulls fed SL had lower (P<0.0001) $CH_4$ production as L/d and L/LW gain than bulls fed F (Table 4).

### 3.3. Ruminal fermentation parameters

Ruminal pH did not differ between bulls fed SL and F (Table 5). Bulls fed SL had lower (P<0.001) ruminal molar concentration of total VFA than bulls fed F. Bulls fed SL had lower (P<0.0001) ruminal proportions of acetate, and the extent of

**Table 4**
Methane production of bulls fed a fibre diet (F) or a diet rich in starch and lipids (SL).

| | P1[a] | | P2 | | P3 | | SEM | P | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | F[b] | SL | F | SL | F | SL | | Diet | Period | Diet*period |
| $CH_4$ (L/d) | 285 a | 228 b | 406 a | 335 b | 506 a | 394 b | 14.9 | <0.0001 | <0.001 | 0.11 |
| $CH_4$/DM intake (L/kg) | 35.4 | 32.5 | 36.3 | 38.8 | 42.6 a | 50.8 b | 2.08 | 0.23 | <0.001 | 0.03 |
| $CH_4$/OM intake (L/kg) | 38.9 | 34.7 | 45.1 | 46.4 | 63.2 | 61.2 | 2.31 | 0.36 | <0.001 | 0.48 |
| $CH_4$ (proportion GE intake) | 0.069 | 0.060 | 0.075 | 0.082 | 0.112 | 0.104 | 0.041 | 0.30 | <0.001 | 0.09 |
| $CH_4$/LW (L/kg) | 0.54 a | 0.45 b | 0.54 a | 0.45 b | 0.54 a | 0.41 b | 0.022 | <0.001 | 0.46 | 0.47 |
| $CH_4$/LW gain (L/kg) | 129 a | 96 b | 202 a | 156 b | 430 a | 301 b | 13.2 | <0.001 | <0.001 | 0.002 |

| 200 first days | F | SL | SEM | P |
|---|---|---|---|---|
| $CH_4$ (L/d)$_{200\,d}$ | 392.2 | 315.2 | 9.10 | <0.001 |
| $CH_4$/LW gain (L/kg) $_{200\,d}$ | 266.3 | 201.7 | 6.60 | <0.001 |

Different letters (a, b) within the same period indicate differences between diets P<0.05.
[a] Period 1–3 (P1–P3): the beginning (24 d), middle (120 d), and end (228 d) of the fattening period of bulls.
[b] F: diet composed of 0.87 concentrate rich in fibre and 0.13 straw, SL: diet composed of 0.87 concentrate rich in starch and fat (from linseed) and 0.13 straw.

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**Table 5**
Rumen fermentative parameters of bulls fed a fibre diet (F) or a diet rich in starch and lipids (SL).

| | P1[a] | | P2 | | P3 | | SEM | P | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | F[b] | SL | F | SL | F | SL | | Diet | Period | Diet*period |
| pH | 6.4 | 6.4 | 6.1 | 6.3 | 6.5 | 6.6 | 0.06 | 0.21 | <0.0001 | 0.41 |
| Total VFA (mM) | 124.3 | 111.3 | 122.2 a | 103.4 b | 166.4 a | 142.7 b | 5.88 | 0.001 | <0.0001 | 0.64 |
| Acetate (mol/100 mol total VFA) | 65.7 a | 54.9 b | 63.6 a | 58.3 b | 69.6 a | 64.7 b | 0.90 | <0.0001 | <0.0001 | 0.001 |
| Propionate (mol/100 mol total VFA) | 18.2 a | 21.6 b | 17.6 a | 21.1 b | 15.5 | 17.0 | 0.55 | <0.0001 | <0.0001 | 0.06 |
| Butyrate (mol/100 mol total VFA) | 12.7 a | 16.5 b | 14.7 | 13.9 | 11.3 | 13.1 | 0.65 | 0.004 | 0.001 | 0.002 |
| Acetate/propionate (mM/mM) | 3.7 a | 2.6 b | 3.6 a | 2.9 b | 4.5 a | 4.0 b | 0.13 | <0.0001 | <0.0001 | 0.02 |

Different letters (a, b) within the same period indicate differences between the diets P<0.05.
[a] Period 1–3 (P1–P3): the beginning (24 d), middle (120 d), and end (228 d) of the fattening of bulls.
[b] F: diet composed of 87% concentrate rich in fibre and 13% straw, SL: diet composed of 87% concentrate rich in starch and fat (from linseed) and 13% straw.

the difference was higher at the beginning of fattening than at other times (P<0.001). Bulls fed SL had higher proportions of propionate (P<0.05) at the beginning and middle of fattening, and higher butyrate (P<0.05) at the beginning of fattening than bulls fed F (Table 5). The acetate to propionate ratio was lower for bulls fed SL than for those fed F and the extent of the difference was higher at the beginning of fattening than at other sampling points (P<0.05).

### 3.4. Feed conversion ratio and relationships between methane production and RFI

Bulls fed SL had higher (P=0.05) LW gain than bulls fed F (Table 3), and bulls fed SL had a lower (P<0.0001) feed conversion ratio when expressed as DM intake/LW gain (Table 3). Over 200 d, there was no relationship between $CH_4$ production as L/d or as L/kg LW gain and $RFI_{200d}$ for bulls fed F or SL.

## 4. Discussion

### 4.1. Methane production

High starch diets, as well as addition of lipid to the diet, are efficient methods of lowering enteric $CH_4$ production (Beauchemin et al., 2009; Martin et al., 2010a). To our knowledge, the combined effects of starch and lipids have not been tested with high concentrate diets over a long term period with a high number of animals. The comparison of a diet high in fibre with a diet higher in starch and lipid aimed to create differences in $CH_4$ production by combining the $CH_4$ suppressing effects of both high concentrate and lipids.

Methane production was high compared to $CH_4$ production predicted by two published equations (Ellis et al., 2009; Sauvant and Giger-Reverdin, 2009). Among the numerous equations available, the one entitled 'P' by Ellis et al. (2009) was chosen because it accounted for DM intake, starch and ADF content of the diet as the main predictors of $CH_4$ produced in the rumen, and had a low mean square error. Methane production (L/d) predicted with this equation for diets F and SL was 249 L/d and 185 L/d, respectively, which is 37 and 42% lower, respectively, than the $CH_4$ production measured using the $SF_6$ technique. Methane expressed relative to GE intake was 37% higher for both diets than $CH_4$ production predicted (*i.e.*, 5.3% GE intake) by the equation of Sauvant and Giger-Reverdin (2009), where level of concentrate in the diet is base predictor of $CH_4$ production. These differences between measured and predicted $CH_4$ values could be due to intake of bedding straw leading to higher $CH_4$ values than expected, but additional straw intake was likely low.

Effects of starch and extruded linseed supplementation on $CH_4$ production resulted in a difference in $CH_4$ production between SL and F when expressed in L/d, but no difference when expressed in L/kg DM intake, except at the end of fattening. The difference in $CH_4$ production between diets is due to DM intake differences. In most studies that included linseed in the diet, $CH_4$ production/kg DM intake was lower when linseed or linseed oil were provided (review by Martin et al., 2010a). The extent of the difference between diets in $CH_4$ production in our study was low (*i.e.*, 20%) compared to the decrease by 47% with starch supplementation (Martin et al., 2007) or by 38% with extruded linseed supplementation (Martin et al., 2008). In our study, the moderate decrease in $CH_4$ production, and the absence of variation in $CH_4$ production/kg DM intake, may be related to lower lipid addition (*i.e.*, 2% of diet DM or ~350 g/d) compared to other experiments. Indeed, Martin et al. (2009) showed a decrease in $CH_4$ production/kg DM intake of 4–6% and 35–37% with supplementation of 2 and 6% of lipid from linseeds, respectively. The decrease in $CH_4$ production could also be due to the moderate increase in starch intake, whereas other studies in beef cattle fed high concentrate diets had higher starch intake (Nkrumah et al., 2006). Finally, the action of starch and lipid on enteric $CH_4$ production may not have been additive, which could also explain the small difference of daily $CH_4$ production between diets.

Mitigation of rumen methanogenesis can be achieved through a decrease in the hydrogen supply to methanogens, by means of a shift of fermentation towards propionate at the expense of acetate and/or by a decrease in protozoans which are hydrogen producers (Morgavi et al., 2010). In our study, the difference in $CH_4$ production between diets F and SL was consistent with the change in VFA concentration and profile. Total VFA concentration was lower for the SL diet, suggesting

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a lower amount of fermented OM in the rumen, which is consistent with a lower OM intake and with substitution of carbohydrate with lipids. Secondly, the acetate:propionate ratio was lower for the SL diet resulting in lower net $H_2$ production. Although lipids from linseeds generally decrease protozoal numbers, this was unlikely in our study because determination of protozoans in rumen contents of the bulls at slaughter found a higher number of protozoa for the SL diet than for the F diet (Popova et al., this issue).

Results show that the difference in $CH_4$ production between diets persisted over the fattening period. Few mitigation strategies lower enteric $CH_4$ over the long term, although this may be the case for starch *versus* fibre, but until now the long term effect of lipid addition on $CH_4$ mitigation has not been documented. Woodward et al. (2006) found that a mixture of linseed and fish oil did not decrease $CH_4$ production by dairy cows after a 3-month period. In contrast, Martin et al. (2010b) showed that $CH_4$ production in dairy cows supplemented with linseed was lower than in unsupplemented counterparts after a year of supplementation. In addition, Grainger et al. (2010) studied effects of whole cottonseed in lactating dairy cows over 12 weeks, and found $CH_4$ emissions/unit added fat were 2.9% lower. Although a part of the difference in our study may be due to differences in DM intake, results suggest that a persistent reduction in $CH_4$ emission is possible with linseed supplementation of beef bulls.

### 4.2. Animal performance, methane and RFI

The lack of a relationship between $CH_4$ and RFI is not consistent with Nkrumah et al. (2006) and Hegarty et al. (2007), who observed a positive relationship between $CH_4$ production and RFI. However in Nkrumah et al. (2006) and Hegarty et al. (2007), animals had been selected for divergent RFI, or chosen from a group which had high variation in RFI, which was not the case in our study. As a result, the range of RFI was lower in our study than in others (Hegarty et al., 2007). Results suggest that the relationship between RFI and $CH_4$ production may be low when animals are not selected for efficiency. More research is needed to study the relationship between RFI and $CH_4$ production in cattle under various dietary conditions.

## 5. Conclusions

Moderate supplementation with extruded linseed combined with high starch decreased $CH_4$ production by 20%, and this reduction persisted throughout the fattening period, even after adjusting $CH_4$ production for LW and LW gain. Overall, bulls fed SL had a lower $CH_4$ production when expressed as L/kg LW or L/kg LW gain. No relationship between $CH_4$ and RFI occurred during the first 200 d of the fattening period. In order to fully assess such a $CH_4$ mitigation strategy, a life cycle assessment analysis should be completed to evaluate the net impact on total GHG emissions when a diet supplemented with starch and fat is fed to fattening bulls.

## Conflict of interest

None.

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