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Fermentation characteristics and methane production of rations with high-lipid feed alternatives *in vitro*

Petra Fant *, Juana C.C. Chagas, Mohammad Ramin

Department of Applied Animal Science and Welfare, Swedish University of Agricultural Sciences, Umeå, 901 83, Sweden

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ABSTRACT

This study evaluated the effects of barley and four oat cultivars differing in lipid content, including one high-oil cultivar, and the replacement of rapeseed meal (RSM) with cold-pressed rapeseed cake (RSC), on *in vitro* ruminal fermentation, degradability, and methane (CH_4) production. An *in vitro* gas production experiment was conducted using a 5 × 3 factorial arrangement of treatments with four 48-h runs. Treatments included a barley mixture [22.6 g crude fat/kg dry matter (DM)] and the oat cultivars Sonja, Niklas, Perttu, and the high-oil oat cultivar Fatima (41.2, 53.7, 58.5, and 81.2 g crude fat/kg DM, respectively), each combined with three levels of RSC (0, 50, and 100 % of protein feed). The basal diet consisted of grass silage (550 g/kg diet DM). Dynamic rumen models were applied to *in vitro* gas data to predict *in vivo* CH_4 production. Predicted CH_4 production (mL/g DM) was 8.3, 9.0, and 12.6 % lower, respectively, for Niklas, Perttu, and Fatima compared with barley ($P \leq 0.01$), and was also lower for Fatima than for Sonja and Niklas ($P \leq 0.01$). Replacing RSM with RSC linearly reduced predicted CH_4 production by 4.3 % ($P \leq 0.01$). *In vitro* ruminal DM and organic matter degradability were lower for all oat cultivars compared with barley ($P \leq 0.01$), and further reduced for Perttu and Fatima compared with Sonja ($P \leq 0.04$). Acetate proportion was higher for Sonja, Niklas, and Perttu than for barley and Fatima ($P \leq 0.02$), while propionate was higher for Perttu and Fatima than for barley, at the expense of butyrate ($P \leq 0.02$). In conclusion, high-oil oats and RSC each reduced predicted *in vivo* CH_4, with additive effects when combined. However, high-oil oats also lowered *in vitro* ruminal degradability. Further *in vivo* studies are required to evaluate effects on digestibility, CH_4 production, and animal performance.

1. Introduction

Ruminants play a vital role in food security by supplying protein through milk and meat for human consumption. However, enteric fermentation of feed in the rumen produces methane (CH_4), a potent greenhouse gas that contributes to climate change [1]. Numerous dietary strategies to mitigate enteric CH_4 emissions from ruminants have been explored. One promising approach is dietary lipid supplementation, which can be achieved by adding lipid sources such as linseed oil [2], or rapeseed oil [3], or by substituting common dietary ingredients with alternatives that have a higher lipid content [4,5].

Among cereal grains, oats (*Avena sativa*) contain more lipid than barley (*Hordeum vulgare*). Replacing barley with oats has been shown to decrease enteric CH_4 production in dairy cows, although much of the reduction is attributed to the lower digestibility of oats [5–7]. The lipid content of previously evaluated oat cultivars ranged from 40 to 55 g crude fat/kg dry matter (DM), although some cultivars contain up to 90 g crude fat/kg DM [8]. These so-called high-oil oat cultivars have not yet been evaluated for their effects on enteric CH_4 production.

Rapeseed meal (RSM) and cold-pressed rapeseed cake (RSC) are by-products of oil extraction from rapeseed (*Brassica napus*). Because of its high crude protein (CP) content, RSM is widely used as a protein feed in many countries. However, RSC contains substantially more lipid (100–200 g crude fat/kg DM) than RSM (40–50 g/kg DM). Partial or complete replacement of RSM with RSC has been shown to decrease enteric CH_4 production without negatively affecting organic matter (OM) digestibility in dairy cows [4,9]. While a recent *in vivo* study [5] investigated the combination of a conventional oat cultivar with RSC, the effects of high-oil oat cultivars, either alone or in combination with RSC, have not yet been studied.

In vitro experiments allow investigation of the underlying fermentation of different feeds. Therefore, the objective of this study was to evaluate the effects of barley and four oat cultivars differing in lipid content, including one high-oil cultivar, combined with varying levels of

* Corresponding author.
E-mail address: petra.fant@slu.se (P. Fant).

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RSC as a replacement for RSM, on *in vitro* ruminal fermentation, degradability, and CH_4 production. Using an *in vitro* gas production system, we tested 15 dietary treatments to assess their potential for CH_4 reduction. We hypothesized that oat cultivars with higher lipid content would produce less CH_4 than barley and low-lipid oat cultivars, and that increasing dietary inclusion of RSC would further reduce CH_4 production.

2. Materials and methods

2.1. Animal ethics

The *in vitro* experiment was carried out in the laboratory at the Department of Applied Animal Science and Welfare, Swedish University of Agricultural Sciences, Umeå, Sweden. All experimental procedures were approved by the Swedish Ethics Committee on Animal Research (Dnr A 6–2021 Umeå, Sweden) and were in accordance with Swedish laws and regulations regarding EU Directive 2010/63/EU on animal research.

2.2. Feeds

The grain cultivars included a mixture of common feed barley cultivars (1), the oat cultivar Sonja (2), the oat cultivar Niklas (3), the oat cultivar Perttu (4), and the high-oil oat cultivar Fatima (5). The barley mixture and Niklas were obtained from Fodercentralen (Sweden), Sonja and Perttu from Boreal Plant Breeding Ltd (Finland), and Fatima from Lantmännen (Sweden). Rapeseed meal was obtained from Fodercentralen and RSC from Säby Gård (Sweden). The grass silage was produced from primary growth perennial leys of timothy (*Phleum pretense*). Prior to incubation, all feeds were dried at 60 °C for 48 h and ground through a 1.0-mm screen (Retsch SM2000; Rheinische, Haan, Germany). The chemical composition of the feeds and the fermentation quality of the silage are presented in Table 1.

2.3. Experimental design and treatments

The *in vitro* gas production experiment consisted of four 48-h runs, during which feed samples were incubated as a total mixed ration (TMR) in buffered rumen fluid. Each run included two replicates per treatment and six blank samples containing only buffered rumen fluid. The experiment followed a 5 × 3 factorial design, comprising five grain treatments (one barley cultivar mixture and four oat cultivars) and three levels of RSM replacement with RSC. The replacement levels were 100 % RSM in protein feed, 50 % RSM and 50 % RSC, and 100 % RSC on a DM basis, with replacements adjusted to be isonitrogenous.

The incubated TMR contained 550 g/kg grass silage, 290–330 g/kg experimental grain, and 120–160 g/kg experimental protein feed (DM basis). A total of 1.00 ± 0.002 g of substrate (DM basis) was weighed into 250-mL glass serum bottles (Schott, Mainz, Germany) to formulate the TMR described above.

2.4. In vitro incubations

Rumen fluid was collected 2 h after morning feeding from two cannulated Nordic Red dairy cows in lactation. The donor cows were fed a TMR of grass silage and concentrate (600:400 g/kg DM) *ad libitum*. Rumen fluid was sampled from the dorsal sac, the intermediate region, and the ventral sac, and filtered through four layers of cheese cloth into two pre-warmed steel thermoses, previously flushed with carbon dioxide (CO_2) to maintain anaerobic conditions. The pH of the rumen fluid was 6.53 ± 0.257 (mean ± SE), measured using a pH meter (744 pH Meter; Metrohm Ltd., Herisau, Switzerland).

The rumen fluid was delivered to the laboratory within 15 min after collection and immediately filtered through four layers of cheesecloth into a measuring cylinder continuously flushed with CO_2. A total of 483 mL of rumen fluid was then transferred through a funnel into another measuring cylinder containing 2415 mL of buffered mineral solution according to Menke [10]. The ratio of buffer solution to rumen fluid in the final buffered rumen fluid was 1:1. Finally, 2 g of peptone (pancreatic digested casein; Merck, Darmstadt, Germany) was added. The buffered rumen fluid was maintained at 39 °C with constant stirring and continuous CO_2 flushing to maintain anaerobic conditions.

To initiate incubation, 60 mL of buffered rumen fluid was added to each of the 36 bottles, which had been flushed with CO_2. Bottles were placed in a water bath at 39 °C and continuously agitated at 40 rpm for 48 h.

2.5. Measurements and sampling

Total gas was measured using the fully automated gas production technique described by Cone et al. [11]. The *in vitro* system recorded total gas volume at 0.2-h intervals, corrected for standard atmospheric pressure (101.3 kPa). Gas samples were collected from each bottle through a rubber suba-seal septum (Z124567–100 EA, 13, Sigma–Aldrich) using a gas-tight syringe (Hamilton, Bonaduz, Switzerland) at 2, 4, 8, 24, and 48 h of incubation. After each sampling, the septum was sealed with Blu Tack (Bostik, Leicester, UK) to maintain an airtight system. Gas samples (0.2 mL) were analyzed for CH_4 concentration using a gas chromatograph (Thermo Scientific™ TRACE 1300™ Series Gas Chromatograph, Thermo Fisher Scientific S.p.A. Milan, Italy) equipped with a thermal conductivity detector. Argon served as the carrier gas at a flow rate of 32 mL/min. Gas peaks were identified by comparison with a standard gas. The standard gas contained 900 mmol/mol CO_2 and 100 mmol/mol CH_4 (AGA Gas AB, Sundbyberg, Sweden). The gas sampling procedure took approximately 35–45 min, ensuring consistent fermentation times across all bottles.

At 48 h of incubation, a 0.5 mL aliquot of residual buffered rumen

Table 1
Chemical composition of feeds used in the *in vitro* gas production experiment (g/kg of DM, unless specified).

Parameter[a]	Grass silage[b]	Barley mixture	Oats Sonja	Oats Niklas	Oats Perttu	Oats Fatima	RSM	RSC
DM (g/kg of fresh weight)	236	897	911	898	918	906	909	921
Ash	76.5	25.6	20.3	28.3	33.1	31.9	75.8	62.9
CP	200	105	109	106	120	129	329	270
aNDFom	512	172	215	269	276	199	323	361
iNDF	95.5	37.9	103	152	152	159	166	140
pdNDF	417	134	112	117	124	40.1	157	221
Starch	14.0	526	513	471	407	349	30.0	22.0
Crude fat	66.6	22.6	41.2	53.7	58.5	81.2	29.7	178

[a] RSM, rapeseed meal; RSC, cold-pressed rapeseed cake; DM, dry matter; CP, crude protein; aNDFom, neutral detergent fiber free of residual ash; iNDF, indigestible NDF; pdNDF, potentially digestible NDF, calculated as NDF – iNDF.
[b] Concentration of ammonia N was 28.8 g/kg N, lactic acid 59.2 g/kg DM, acetic acid 11.7 g/kg DM, propionic acid 1.0 g/kg DM, butyric acid 0.3 g/kg DM, and pH 3.93.

fluid was collected from each bottle for volatile fatty acid (VFA) analysis. Samples were pooled by treatment, combined, and stored in Eppendorf tubes at −18 °C until analysis. The pH of each bottle was also measured using a 744 pH Meter (Metrohm Ltd., Herisau, Switzerland).

2.6. *In vitro* ruminal degradability

In vitro ruminal degradability was assessed as described by Fant and Ramin [12]. Briefly, the incubation residue from each bottle was transferred to pre-weighed 07–11/5 Sefar Petex *in situ* nylon bags (11 μm pore size; Sefar AG, Heiden, Switzerland), according to Krizsan et al. [13]. Excess liquid was removed by filtration through the bag pores. To determine *in vitro* true DM degradability (DMD) and organic matter degradability (OMD), the nylon bags containing the residues were boiled for 1 h in a neutral detergent solution supplemented with heat-stable α-amylase and sodium sulfite (Na_2SO_3). The bags were then dried at 60 °C for 48 h and weighed to calculate *in vitro* DMD (g/kg DM) according to the following equation:

DMD = [Incubated DM (g) – neutral detergent fiber (NDF) residue corrected for blank (g)] / Incubated DM (g) × 1000

In vitro OMD (g/kg OM) was determined by combustion of the incubation residues (excluding bags) at 500 °C for 4 h, and calculated according to the following equation:

OMD = [Incubated OM (g) – NDF residue corrected for blank and ash (g)] / Incubated OM (g) × 1000

2.7. Chemical analysis

All feed samples (grass silage, cereal grains, RSM, and RSC) were analyzed for DM, ash, crude fat, CP, neutral detergent fiber free of residual ash (aNDFom), indigestible NDF (iNDF), and starch. Dry matter concentration was determined by oven drying at 105 °C for 16 h, followed by ash determination through combustion at 500 °C for 4 h [14]. Crude fat concentration was assessed through ether extraction and HCl-hydrolysis according to AOAC method 954.02 [14], and starch was analyzed with a YSI Analyzer (YSI 2950D-1 Biochemistry Analyzers) at the Dairy One Forage Laboratory (Ithaca, NY, USA). Total nitrogen concentration was assessed using the Kjeldahl method, and CP concentration was calculated as total nitrogen × 6.25.

The aNDFom concentration was determined according to Mertens [15], using heat stable α-amylase and sodium sulfite in an ANKOM200 digestion unit (ANKOM Technology Corp., Macedon, NY, USA), expressed exclusive of residual ash. Indigestible NDF concentration was assessed according to Krizsan et al. [13]. Briefly, 2 g of feed samples were placed in 11 μm pore size nylon bags and incubated for 288 h in three rumen-cannulated lactating dairy cows (one replicate per cow) fed a TMR of grass silage and concentrate (600:400 g/kg DM) *ad libitum*. Indigestible NDF concentration was expressed exclusive of residual ash.

Silage samples were initially frozen, then thawed and compressed. Silage juice was diluted 1:1 with distilled water for analysis of ammonia nitrogen using a Kjeltec 2100 Distillation Unit (Foss Analytical Ltd.) and for pH measurement. Lactic acid and VFA concentrations were analyzed as outlined by Ericson and André [16], and silage DM was adjusted for volatile losses as described by Huida et al. [17]. Volatile fatty acid concentrations in residual buffered rumen fluid were measured by high-performance liquid chromatography using a Waters Acquity system (Waters, Milford, MA) following the procedure of Puhakka et al. [18].

2.8. Calculations

In vivo CH_4 production was predicted from data obtained in the *in vitro* gas production experiment, following the method of Ramin and Huhtanen [19]. At each 0.2-h interval, cumulative CH_4 production (V_{CH4}) was calculated as:

$$V_{CH4}\ (\text{mL}) = V_{HS}\ (\text{mL}) \times CH_4\ (\text{mL/mL}) + V_{GP}\ (\text{mL}) \times A \times CH_4\ (\text{mL/mL}),$$

where V_{HS} represents the headspace volume, CH_4 represents the CH_4 concentration in the headspace; V_{GP} is the gas production volume, and A is a coefficient (0.55) that reflects the ratio of CH_4 concentration in outflow gas to the headspace, derived from a mechanistic model [19]. Methane concentration at each 0.2-h interval was estimated by fitting a logarithmic regression to the measured CH_4 values collected at five time points.

To estimate kinetic parameters for total gas and CH_4 production, data from the 0.2-h intervals were fitted to the two-pool Gompertz model of Schofield et al. [20]:

$$V_t = V_1 \times \text{Exp}\{-\text{Exp}[1 - k_1 \times (t - L_1)]\} + V_2 \times \text{Exp}\{-\text{Exp}[1 - k_2 \times (t - L_2)]\},$$

where Vt is the total gas or CH_4 volume at time t; V_1, k_1, and L_1 represent the asymptotic cumulative gas production (mL/g of DM), rate constant (1/h), and lag time (h) for the rapid pool, respectively; V_2, k_2, and L_2 represent the same parameters for the slower pool. Modeling was performed using the NLIN procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC).

To predict the proportion of asymptotic CH_4 production occurring during feed residence in the rumen, the kinetic parameters were further applied to a dynamic, mechanistic two-pool rumen model [21], with modifications described by Ramin and Huhtanen [19]. Simulations assumed a mean rumen retention time of 50 h, representing intake level at maintenance. Predicted *in vivo* CH_4 production (mL/g of DM) was calculated as CH_4 = proportion of asymptotic CH_4 production × asymptotic CH_4 production (mL/g of DM).

Volatile fatty acid concentrations (mmol/L) in rumen fluid residues were calculated by subtracting the average VFA concentration in blank samples from that in treatment samples. Total VFA production (mmol/g DM) was determined by multiplying this difference (sample − blank) by the sample volume (60 mL).

2.9. Statistical analysis

All data were tested for normality using the Shapiro-Wilk test and for homoscedasticity of residuals prior to statistical analysis. Data on total gas and predicted *in vivo* CH_4 production, *in vitro* ruminal degradability, and pH were analyzed by ANOVA using the MIXED procedure in SAS according to the following model:

$$Y_{ijkl} = \mu + G_i + L_j + R_k + b_l + G_i \times L_j + \varepsilon_{ijkl}$$

where Y_{ijkl} is the dependent variable, μ is the overall mean, G_i is the fixed effect of cultivar ($i = 5$), L_j is the fixed effect of RSC inclusion level ($j = 3$), R_k is the fixed effect of run ($k = 4$), b_l is the random effect of bottle (position in the water bath), $G_i \times L_j$ is the interaction effect between cultivar and RSC inclusion level, and ε_{ijkl} is the random residual error. Because samples for total VFA production and VFA molar proportions were pooled by treatment within each *in vitro* run ($n = 4$), these data were analyzed according to the following model:

$$Y_{ijk} = \mu + G_i + L_j + r_k + G_i \times L_j + \varepsilon_{ijk}$$

where Y_{ijk} is the dependent variable, μ is the overall mean, G_i is the fixed effect of cultivar ($i = 5$), L_j is the fixed effect of RSC inclusion level ($j = 3$), r_k is the random effect of run ($k = 4$), $G_i \times L_j$ is the interaction effect between cultivar and RSC inclusion level, and ε_{ijk} is the random residual error.

Least square means were obtained using the LSMEANS statement in SAS. Differences were declared significant at $P < 0.05$, and $0.05 < P < 0.10$ was considered a tendency. When the overall P-value for cultivar was significant, Tukey's test was used for multiple comparisons. Linear and quadratic orthogonal contrasts were included to assess the effect of RSC inclusion level.

3. Results

3.1. Chemical composition of experimental diets

The chemical composition of the experimental diets is presented in Table 2. The diet containing the high-oil oat cultivar Fatima with 100 % RSC had the highest numerical crude fat concentration (88.7 g/kg DM), while the diet containing the barley mixture with 100 % RSM had the lowest (47.7 g/kg DM). The aNDFom concentration ranged from 377 to 419 g/kg DM, with the highest value observed in the diet with Perttu and 100 % RSC and the lowest in the barley mixture with 100 % RSM. The iNDF concentration ranged from 85.0 to 125 g/kg DM, with the highest value in the diet with Fatima and 100 % RSM and the lowest with the barley mixture and 100 % RSM. The average CP concentration across all diets was 187 g/kg DM, with minimal variation (2.91 g/kg DM), ensuring consistent nitrogen levels.

3.2. Predicted total gas and methane production

Predicted *in vivo* total gas production (mL/g DM) was lower for the oat cultivars Niklas, Perttu, and Fatima compared with the barley mixture ($P \leq 0.01$), with Fatima producing less total gas than the oat cultivar Sonja ($P \leq 0.01$; Table 3). Predicted *in vivo* CH_4 production (mL/g DM) was lower for Niklas, Perttu, and Fatima than for the barley mixture and Sonja ($P \leq 0.01$) and it was also lower for Fatima compared with Niklas ($P = 0.04$). Replacing RSM with RSC resulted in a slight linear reduction in predicted *in vivo* CH_4 production ($P \leq 0.01$). Additionally, an interaction between cultivar and RSC inclusion level was observed for the CH_4 production rate ($P = 0.01$; Table 3).

3.3. In vitro ruminal degradability and fermentation

In vitro ruminal OMD was lower for all oat cultivars compared with the barley mixture ($P < 0.01$; Table 4). Moreover, OMD was lower for Perttu and Fatima than for Sonja ($P \leq 0.04$), and lower for Fatima than for Niklas ($P < 0.01$). Replacing RSM with RSC caused a slight linear reduction in OMD ($P = 0.01$; Table 4). Cultivar affected total VFA production (mmol/g DM; $P = 0.01$), but after correction with Tukey's test, only tendencies were observed ($P \geq 0.07$; Table 4).

The molar proportion of acetate (mmol/mol VFA) was higher for Sonja, Niklas, and Perttu, than for the barley mixture and Fatima ($P \leq 0.02$). Perttu and Fatima had higher propionate proportions than the barley mixture ($P \leq 0.02$), with Fatima exceeding Sonja and Niklas ($P < 0.01$). The molar proportion of butyrate was lower for all oat cultivars compared with the barley mixture ($P < 0.01$) and was lower for Fatima than for Sonja and Niklas ($P \leq 0.01$). Replacing RSM with RSC led to a linear reduction in acetate and a linear increase in butyrate molar proportions ($P < 0.01$; Table 4).

4. Discussion

4.1. Total gas and methane production

Predicted *in vivo* CH_4 production (mL/g DM) was 8.3, 9.0, and 12.6 % lower for the oat cultivars Niklas, Perttu, and Fatima, respectively, compared with the barley mixture. This reduction may be primarily explained by the lower *in vitro* ruminal OMD of these cultivars. Since enteric CH_4 is produced from digested matter, decreased ruminal degradability is expected to reduce CH_4 production [22]. However, the observed reductions in *in vitro* OMD were relatively small (2.6, 3.1, and 4.0 % for Niklas, Perttu, and Fatima, respectively), suggesting involvement of additional CH_4 mitigating mechanisms. Moreover, when expressed relative to *in vitro* OMD (mL/g digested OM), predicted *in vivo* CH_4 production was 4–5 % lower for Perttu and Fatima compared with barley, although this difference was not statistically significant ($P = 0.11$).

A minor contributor to the observed CH_4 reduction (mL/g DM) could be the higher dietary lipid content, particularly in the high-oil oat cultivar Fatima. Dietary lipid supplementation is well known to decrease enteric CH_4 production [23]. In this study, fermentable substrate (mainly starch) was partially replaced with non-fermentable fatty acids, reducing hydrogen availability in the rumen. As the ratio of CH_4 to total gas was unaffected by cultivar, this suggests that the effect of lipids was primarily due to reduced fermentation and hydrogen availability. Notably, the high-oil cultivar Fatima showed 9.9 and 4.8 % lower predicted *in vivo* CH_4 production compared with the oat cultivars Sonja and Niklas, respectively. These findings indicate that differences in CH_4 production potential exist not only between cereal species but also among cultivars differing in fiber and oil content.

In the case of replacing RSM with RSC, the decrease in CH_4 production can be primarily attributed to the higher crude fat content of RSC, as effects on OMD were small. These results are consistent with previous studies. An *in vitro* study by García-Rodríguez et al. [24] reported a 6–12 % decrease in CH_4 production (mmol/d) depending on the level of RSC inclusion in the concentrate. In our study, predicted *in vivo* CH_4 production (mL/g DM) was 4.3 % lower with the RSC100 diet compared with the RSM100 diet, corresponding to a 1.9 % decrease per percentage of crude fat added by RSC. Although it is difficult to compare *in vitro* results with the results of *in vivo* studies due to the differences in environments, *in vivo* studies have reported a wide range of responses to

Table 2
Chemical composition of the experimental diets (g/kg of DM, unless specified).

Parameter[a]	Treatment[b]														
	Barley mixture			Oats Sonja			Oats Niklas			Oats Perttu			Oats Fatima		
	RSM 100	RSM 50	RSC 100	RSM 100	RSM 50	RSC 100	RSM 100	RSM 50	RSC 100	RSM 100	RSM 50	RSC 100	RSM 100	RSM 50	RSC 100
DM (g/kg of fresh weight)	883	884	885	885	886	887	884	885	886	895	895	895	904	904	903
Ash	59.6	59.7	59.6	57.9	58.1	58.0	60.5	60.6	60.3	62.1	62.0	61.7	61.7	61.7	61.4
CP	184	185	184	186	186	185	184	185	184	189	189	188	192	192	190
aNDFom	377	383	389	391	396	402	409	413	417	411	415	419	386	391	397
iNDF	85.0	85.7	85.9	106	106	105	123	121	119	123	121	119	125	123	121
pdNDF	292	297	304	285	291	297	287	292	299	289	294	301	261	268	276
Starch	185	174	164	181	170	160	167	157	148	146	138	129	126	120	112
Crude fat	47.7	58.2	71.7	53.8	63.9	77.1	57.9	67.8	80.7	59.5	69.3	82.1	67.0	76.3	88.7

[a] DM, dry matter; CP, crude protein; aNDFom, neutral detergent fiber free of residual ash; iNDF, indigestible NDF; pdNDF, potentially digestible NDF, calculated as NDF − iNDF.
[b] RSM100, 100 % rapeseed meal in protein feed; RSM50, 50 % rapeseed meal and 50 % cold-pressed rapeseed cake in protein feed; RSC100, 100 % cold-pressed rapeseed cake in protein feed (120–160 g/kg of diet DM).

Table 3
Effects of cultivar (barley and four oat cultivars) and level of cold-pressed rapeseed cake on predicted *in vivo* total gas and methane production.

Parameter[a]	Treatment[b]															SEM[c]	*P*-value[d]			
	Barley mixture			Oats Sonja			Oats Niklas			Oats Perttu			Oats Fatima				C	Lin	Quad	C × L
	RSM 100	RSM 50	RSC 100	RSM 100	RSM 50	RSC 100	RSM 100	RSM 50	RSC 100	RSM 100	RSM 50	RSC 100	RSM 100	RSM 50	RSC 100					
Total gas																				
Asymptotic gas (mL/g DM)	289[a]	284[a]	259[a]	278[a,b]	270[a,b]	263[a,b]	264[b]	256[b]	256[b]	255[b]	261[b]	254[b]	261[c]	228[c]	242[c]	8.2	<0.01	<0.01	0.55	0.10
Rate (1/h)	0.101	0.097	0.124	0.105	0.106	0.119	0.094	0.107	0.117	0.120	0.107	0.109	0.099	0.121	0.142	0.014	0.57	0.02	0.43	0.66
Predicted gas (mL/g DM)	265[a]	259[a]	240[a]	255[a,b]	248[a,b]	245[a,b]	238[b,c]	236[b,c]	239[b,c]	237[b,c]	241[b,c]	232[b,c]	239[c]	222[c]	227[c]	7.6	<0.01	0.01	0.93	0.45
CH_4																				
Asymptotic CH_4 (mL/g DM)	54.0[a]	50.8[a]	48.2[a]	51.2[a,b]	48.8[a,b]	47.9[a,b]	49.4[b,c]	45.2[b,c]	45.1[b,c]	43.6[c]	46.3[c]	45.5[c]	46.0[c]	43.2[c]	42.5[c]	1.65	<0.01	<0.01	0.50	0.12
Rate (1/h)	0.065	0.067	0.069	0.067	0.068	0.070	0.064	0.070	0.071	0.079	0.069	0.069	0.067	0.069	0.076	0.0029	0.12	0.09	0.40	0.01
Predicted CH_4 (mL/g DM)	46.1[a]	43.6[a]	41.5[a]	43.9[a]	41.9[a]	41.4[a]	41.7[b]	39.1[b]	39.2[b]	38.4[b,c]	40.0[b,c]	39.1[b,c]	39.5[c]	37.0[c]	37.2[c]	1.19	<0.01	<0.01	0.40	0.26
Predicted CH_4 (mL/g DM digested)	49.3	48.0	45.4	50.2	49.7	45.5	47.7	44.7	43.9	42.4	46.2	48.0	44.6	47.3	45.3	2.07	0.12	0.24	0.30	0.08
Predicted CH_4 (mL/g OM digested)	48.8	47.7	44.9	49.7	49.3	45.0	47.2	44.1	43.4	41.9	45.8	47.4	44.1	46.8	44.7	2.50	0.11	0.23	0.30	0.10
CH_4/Total gas	0.174	0.168	0.175	0.172	0.169	0.169	0.176	0.165	0.164	0.165	0.167	0.169	0.165	0.170	0.164	0.0042	0.29	0.36	0.45	0.39

[a] DM, dry matter; OM, organic matter; Predicted gas and CH_4, predicted *in vivo* total gas and CH_4 production based on observed values corrected for a mean ruminal retention time of 50 h; CH_4/Total gas, predicted *in vivo* CH_4 (mL/g DM) divided by predicted *in vivo* total gas (mL/g DM).
[b] RSM100, 100 % rapeseed meal in protein feed; RSM50, 50 % rapeseed meal and 50 % cold-pressed rapeseed cake in protein feed; RSC100, 100 % cold-pressed rapeseed cake in protein feed (120–160 g/kg diet DM).
[c] SEM, standard error of the mean.
[d] Within a row, cultivar means without a common superscript differ significantly (Tukey's test, $P < 0.05$). C, main effect of cultivar; Lin, linear effect of RSC level; Quad, quadratic effect of RSC level; C × L, interaction effect between cultivar and RSC level.

Table 4
Effects of cultivar (barley and four oat cultivars) and level of cold-pressed rapeseed cake on *in vitro* ruminal degradability, pH, and fermentation pattern.

Parameter[a]	Treatment[b]															SEM[c]	*P*-value[d]			
	Barley mixture			Oats Sonja			Oats Niklas			Oats Perttu			Oats Fatima				C	Lin	Quad	C × L
	RSM 100	RSM 50	RSC 100	RSM 100	RSM 50	RSC 100	RSM 100	RSM 50	RSC 100	RSM 100	RSM 50	RSC 100	RSM 100	RSM 50	RSC 100					
DMD (g/kg DM)	893^{a}	891^{a}	886^{a}	867^{b}	873^{b}	871^{b}	$869^{b,c}$	$865^{b,c}$	$859^{b,c}$	$863^{c,d}$	$863^{c,d}$	$854^{c,d}$	858^{d}	854^{d}	846^{d}	6.4	<0.01	0.01	0.25	0.75
OMD (g/kg OM)	901^{a}	898^{a}	895^{a}	875^{b}	881^{b}	880^{b}	$879^{b,c}$	$876^{b,c}$	$869^{b,c}$	874^{c}	871^{c}	864^{c}	867^{c}	863^{c}	856^{c}	7.0	<0.01	0.01	0.36	0.63
pH	6.27^{a}	6.29^{a}	6.29^{a}	$6.29^{a,b}$	$6.30^{a,b}$	$6.32^{a,b}$	$6.30^{a,b}$	$6.31^{a,b}$	$6.34^{a,b}$	$6.32^{a,b}$	$6.30^{a,b}$	$6.34^{a,b}$	6.33^{b}	6.33^{b}	6.35^{b}	0.063	0.01	0.05	0.32	0.99
VFA production (mmol/g DM)	6.30	5.99	6.27	6.06	6.44	6.18	5.93	6.01	5.33	5.51	5.79	5.67	5.96	5.79	5.22	0.292	0.01	0.18	0.26	0.44
VFA molar proportions (mmol/mol)																				
Acetate	594^{a}	592^{a}	588^{a}	604^{b}	598^{b}	597^{b}	601^{b}	596^{b}	596^{b}	599^{b}	596^{b}	594^{b}	593^{a}	593^{a}	592^{a}	5.4	<0.01	<0.01	0.28	0.74
Propionate	229^{a}	233^{a}	233^{a}	232^{a}	232^{a}	233^{a}	$231^{a,b}$	$234^{a,b}$	$235^{a,b}$	$237^{b,c}$	$236^{b,c}$	$235^{b,c}$	239^{c}	239^{c}	240^{c}	4.0	<0.01	0.15	0.84	0.68
Butyrate	132^{a}	133^{a}	134^{a}	123^{b}	125^{b}	126^{b}	123^{b}	125^{b}	126^{b}	$122^{b,c}$	$124^{b,c}$	$126^{b,c}$	122^{c}	122^{c}	123^{c}	2.3	<0.01	<0.01	0.70	0.74
Isobutyrate	8.90	8.32	9.17	8.80	9.39	9.87	9.69	9.36	9.29	8.92	9.46	9.62	10.03	9.86	9.33	0.551	0.10	0.48	0.71	0.40
Valerate	22.6^{a}	21.9^{a}	21.6^{a}	19.2^{b}	20.8^{b}	19.2^{b}	20.4^{b}	20.2^{b}	19.6^{b}	19.8^{b}	19.7^{b}	20.1^{b}	$20.2^{a,b}$	$21.5^{a,b}$	$21.0^{a,b}$	0.79	<0.01	0.81	0.26	0.67
Isovalerate	13.4	12.5	14.1	13.9	15.0	14.9	15.3	15.0	14.0	13.0	15.1	14.8	15.6	15.4	15.1	1.38	0.21	0.63	0.71	0.79
Acetate/propionate	$2.59^{a,b}$	$2.55^{a,b}$	$2.53^{a,b}$	2.61^{a}	2.58^{a}	2.57^{a}	$2.60^{a,b}$	$2.55^{a,b}$	$2.54^{a,b}$	2.53^{b}	2.53^{b}	2.53^{b}	2.48^{c}	2.49^{c}	2.47^{c}	0.066	<0.01	0.02	0.52	0.67

[a] DM, dry matter; DMD, dry matter degradability; OMD, organic matter degradability; pH, measured at 48 h of incubation; VFA, volatile fatty acids.
[b] RSM100, 100 % rapeseed meal in protein feed; RSM50, 50 % rapeseed meal and 50 % cold-pressed rapeseed cake in protein feed; RSC100, 100 % cold-pressed rapeseed cake in protein feed (120–160 g/kg diet DM).
[c] SEM, standard error of the mean.
[d] Within a row, cultivar means without a common superscript differ significantly (Tukey's test, $P < 0.05$). C, main effect of cultivar; Lin, linear effect of RSC level; Quad, quadratic effect of RSC level; C × L, interaction effect between cultivar and RSC level.

rapeseed lipid supplementation. Similar to our results, Moate et al. [25] found a 1.9 % decrease in CH_4 yield (g/kg of DM intake) for each percentage of lipid added in the form of cold-pressed canola meal, whereas Brask et al. [9] reported an average of 4.4 % decrease in CH_4 yield for every percentage of dietary lipid added in different forms of rapeseed, including rapeseed cake.

4.2. *In vitro ruminal degradability and fermentation*

In agreement with previous *in vitro* [6,26] and *in vivo* studies [5,7,27], OMD was lower for all oat cultivars compared with barley. The differences in *in vitro* ruminal OMD between the oat cultivars and the barley mixture, as well as among the oat cultivars themselves, can largely be explained by variations in dietary iNDF content and by variations in the dietary NDF-to-starch ratio [28]. Additionally, the higher crude fat content in oat cultivars compared with barley, and among the oat cultivars, may have contributed to reduced ruminal OMD by impairing the activity of cellulolytic bacteria responsible for fiber degradation [29,30].

Interestingly, molar proportion of propionate was higher for the high-oil oat cultivar Fatima than for barley and the oat cultivars Sonja and Niklas, resulting in a lower acetate-to propionate ratio, which is associated with reduced CH_4 production [31]. This shift was accompanied by a lower proportion of butyrate. Since acetate and butyrate production generates hydrogen, whereas propionate formation acts as a hydrogen sink, these results indicate a shift in the balance between hydrogen-producing and hydrogen utilizing-VFA pathways [31], consistent with the reduction in CH_4.

The slight reduction (−0.7 %) in *in vitro* ruminal OMD observed when replacing RSM with RSC is likely due to the higher crude fat content of RSC (57.2 vs. 80.0 g crude fat/kg diet DM in RSM100 and RSC100, respectively). Fiber content, particularly iNDF, was similar across the different RSC inclusion levels (112 vs. 110 g iNDF/kg DM in RSM100 and RSC100, respectively), indicating that fiber was not a contributing factor. The reduction is minor and unlikely to be of biological significance, especially as lower ruminal degradability may be compensated for in post-ruminal digestion processes [32]. Consistent with this interpretation, Brask et al. [9] and Bayat et al. [4] reported no effect of replacing RSM with RSC on *in vivo* apparent total-tract OM digestibility.

Given the minor effect of increased dietary RSC inclusion on *in vitro* ruminal OMD, similar total VFA production (mmol/g DM) across different inclusion levels is expected. The modest linear decrease in acetate proportion (0.7 %) resulted in a reduction (−1.4 %) in the acetate-to-propionate ratio, a shift which is generally associated with reduced enteric CH_4 production [33]. However, this decrease was accompanied by an increase in butyrate and no change in propionate proportion. Notably, Ungerfeld et al. [31] showed that inhibiting CH_4 redirected hydrogen toward propionate, but not toward butyrate. Although RSC significantly affected VFA molar proportions, the magnitude of these effects was small, and the observed shifts in fermentation are unlikely to explain the reduction in CH_4. This is further supported by the findings of Bayat et al. [4], who reported no effects on the major VFA when replacing RSM with RSC *in vivo*.

4.3. *Method strengths and limitations*

In vitro gas production experiments provide a useful first step for screening diets for their CH_4 production potential. This approach is cost-effective, requires relatively little labor, and reduces the need for experimental animals. However, the *in vitro* system and the values it produces do not account for the dynamics of ruminal digestion, which play a critical role in CH_4 production. To address this limitation, Ramin and Huhtanen [19] developed a model that incorporates rumen dynamics, such as passage rate, to predict *in vivo* CH_4 production. This model was applied in the present study, as it has been shown to be in good agreement with measured *in vivo* values [34].

Danielsson et al. [34] evaluated 49 diets with varying nutrient compositions and compared predicted *in vivo* CH_4 production with values measured in respiration chambers or with the GreenFeed system (C-Lock Inc., Rapid City, SD). The predictions showed strong agreement with measured values ($R^2 = 0.96$) with a relatively low prediction error (9.5 % of observed mean). Residual analysis indicated that the model performed well across diets differing in DM intake, digestibility, and dietary concentrations of fat and starch, but less well across diets differing in concentrate proportions. Under the conditions of the current experiment, the model is appropriate, as most of the CH_4-mitigating effect was driven by digestibility and crude fat, with concentrate inclusion levels being similar across treatments.

Recently, Fant and Ramin [12] compared predicted *in vivo* CH_4 production with GreenFeed measurements for barley and oat diets and found that the predicted diet rankings matched the measured values, supporting the validity of the model. Nevertheless, it is important to note that the prediction method is standardized to maintenance-level of feed intake, which does not reflect feed intake levels typical of lactating cows. Using a 35-h rumen retention time (lactation) improved R^2 and reduced error compared with 50-h [12]. Therefore, while the *in vitro* system and CH_4 prediction model are valuable for initial evaluation of feeds and diets [35], further validation of high-oil oats and RSC under production-level intake conditions is recommended.

5. Conclusion

Compared with barley, the oat cultivars Niklas, Perttu, and the high-oil cultivar Fatima reduced predicted *in vivo* methane production by 8.3, 9.0, and 12.6 %, respectively, while also reducing *in vitro* organic matter degradability by 2.6, 3.1, and 4.0 %, respectively. Furthermore, Fatima reduced predicted methane production by 4.8 and 9.9 % compared with the oat cultivars Niklas and Sonja, respectively. Replacing rapeseed meal with cold-pressed rapeseed cake reduced predicted methane production by 4.3 %, irrespective of cultivar. Although methane production was predicted from measured *in vitro* gas data using a validated modeling approach, further *in vivo* research is required to evaluate effects on feed intake, production performance, and methane emissions under practical feeding conditions.

CRediT authorship contribution statement

Petra Fant: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Juana C.C. Chagas:** Writing – review & editing, Funding acquisition, Conceptualization. **Mohammad Ramin:** Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors state that they have no conflicts of interest.

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Data availability

Data will be made available on request.

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