Effects of different barley and oat varieties on methane production, digestibility, and fermentation pattern in vitro

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ABSTRACT

The objective of this in vitro study was to determine the effects of different barley and oat varieties on CH₄ production, digestibility, and rumen fermentation patterns in dairy cows. Our hypothesis was that oat-based diets would decrease CH₄ production compared with barley-based diets, and that CH₄ production would differ between varieties within grain species. To evaluate this hypothesis, we conducted an in vitro experiment using a fully automated gas production technique, in which the total gas volume was automatically recorded by the system. The experiment consisted of triplicate 48-h incubations with 16 treatments, including 8 different varieties of each grain. The grain varieties were investigated as a mix with an early-cut grass silage (1:1 ratio of grain to silage on a dry matter basis) and mixed with buffered rumen fluid. We estimated predicted in vivo total gas production and CH₄ production by applying a set of models to the gas production data obtained by the in vitro system. We also evaluated in vitro digestibility and fermentation characteristics. The variety of grain species did not affect total gas production, CH₄ production, or fermentation patterns in vitro. However, in vitro-determined digestibility and pH were affected by variety of grain species. Grain species affected total gas and CH₄ production: compared with barley-based diets, oat-based diets decreased total gas production and CH₄ production by 8.2 and 8.9%, respectively, relative to dry matter intake. Grain species did not affect CH₄ production relative to in vitro true dry matter digestibility. Oat-based diets decreased digestibility and total volatile fatty acid production, and maintained a higher pH at 48 h of incubation compared with barley-based diets. Grain species did not affect fermentation patterns, except for decreased molar proportions of valerate with oat-based diets. These results

suggest that replacing barley with oats in dairy cow diets could decrease enteric CH₄ production.

Key words: oats, barley, methane, in vitro

INTRODUCTION

After water vapor and CO₂, methane (CH₄) is the third most important greenhouse gas contributing to climate change. Compared with CO₂, CH₄ is characterized by a greater efficiency in absorbing heat energy and a shorter atmospheric lifetime (12–13 yr), resulting in a global warming potential 28 times greater than CO₂ (IPCC, 2014). Ruminant livestock account for approximately 37% of the global anthropogenic CH₄ emissions; a greater part of that originates from enteric fermentation, and a lesser part from manure (FAO, 2006). In addition, enteric CH₄ production represents an energy loss to the animal, ranging from 2 to 12%, depending on diet composition and feeding level (Johnson and Johnson, 1995). For these 2 reasons, research aimed at mitigating enteric CH₄ emissions from ruminants is very important.

Enteric CH₄ production in ruminants is affected by DMI (Johnson and Johnson, 1995), diet composition, chemical composition of feed components, diet digestibility, and individual animal characteristics (Yan et al., 2000). Dietary strategies to decrease CH₄ emissions from ruminants have been widely investigated. Many of these involve dietary supplements, such as ionophores (Guan et al., 2006), nitrate (van Zijderveld et al., 2011), secondary plant compounds (Bhatta, 2015), 3-nitroxypentanol (Hristov et al., 2015), and oil supplements (Martin et al., 2008; Beauchemin et al., 2009), which have all been found effective to various degrees. However, for farmers to adopt a CH₄-mitigating dietary strategy, the strategy has to be safe, practically applicable at a farm scale, and economically beneficial to the farmer (Hristov et al., 2013), posing problems for the practical application of the strategies above.

Oats (*Avena sativa*) and barley (*Hordeum vulgare*) are common grains in the diet of dairy cows, especially

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in the Nordic countries, because yields are relatively high and moderate input is required. Many studies suggest that replacing barley with oats in the diet of dairy cows increases milk yield, decreases milk protein and fat concentrations, and improves the composition of milk fat by increasing the concentration of unsaturated fatty acids (Martin and Thomas, 1988; Ekern et al., 2003; Vanhatalo et al., 2006). In terms of chemical composition, oats express a greater concentration of oil and fiber, with a greater proportion of soluble β -glucans than barley; barley expresses a higher concentration of starch (Lee et al., 1997). In addition, oats contain several types of antioxidants, such as phenolic compounds and avenanthramides, which are not present in barley (Peterson, 2001). All of these differences in chemical composition between oats and barley could influence enteric CH_4 production.

The first objective of this study was to compare oats and barley (and different varieties of each grain) in a grass-silage-based diet in terms of CH_4 production, digestibility, and fermentation patterns using an in vitro gas production system. Our hypothesis was that replacing barley with oats would decrease CH_4 production, and that CH_4 production would differ between varieties of grain species. The second objective was to conduct a regression analysis to explain possible mechanisms of the differences in CH_4 production and compare observed responses to the model-predicted responses as a means of evaluating whether oats have a specific CH_4 mitigation effect.

MATERIALS AND METHODS

Grain Samples

The experimental feed material consisted of 8 varieties of oats and 8 varieties of barley. All grains were hulled and obtained as seed samples from Boreal Plant Breeding Ltd. (Jokioinen, Finland). The grain samples (including hull) were dried at 60°C in a forced-air oven for 48 h and milled through a 1.0-mm screen using a laboratory mill (Retsch SM2000; Rheinische, Haan, Germany).

Experimental Design and Treatments

The in vitro gas production experiment consisted of triplicate runs of 48 h in vitro. Each run included 36 samples: 16 treatments $\times$ 2 replicates and 4 blank samples containing only buffered rumen fluid. Feed samples and buffered rumen fluid were mixed in serum bottles (250 mL; Schott, Mainz, Germany) and incubated in a water bath. Before each run, serum bottles were ran-

domized to the 36 positions in the water bath so that each treatment had a different position during each run. The ratio of incubated grain to silage was 1:1 on a DM basis; each treatment contained 500 mg of barley or oats, and 500 mg of silage. The silage was early-cut silage harvested from primary growth of a third-year lay dominated by timothy (*Phleum pratense*). The fields were fertilized with 70 kg of N/ha in the spring. The silage samples were treated in the same way as the grain samples.

In Vitro Incubations

All experimental procedures were approved by the Umeå Ethics Committee for Animal Research, Sweden, and by the National Animal Ethics Committee, Finland. Rumen fluid was collected approximately 2 h after morning feeding from 2 fistulated Nordic Red dairy cows in late lactation that were fed a TMR of grass silage and concentrate (600:400 g/kg on a DM basis) ad libitum. The concentrate consisted of rolled barley and rape seed meal (800:200 g/kg on a DM basis). The rumen fluid was kept in 2 steel thermoses that had been prewarmed and flushed with CO_2 to ensure an anaerobic environment. The pH value of the rumen fluid (mean 6.27, standard deviation 0.12) was recorded (744 pH Meter; Metrohm Ltd., Herisau, Switzerland) before it was filtered through 4 layers of cheesecloth into a measuring cylinder continuously flushed with CO_2 . A total of 483 mL of rumen fluid was transferred through a funnel into another measuring cylinder containing 483 mL of buffer solution mixed with micro- and macrominerals as described by Menke (1988), at 39°C under constant stirring and continuous flushing with CO_2 . The buffered rumen fluid was supplemented with peptone (pancreatic digested casein; Merck, Darmstadt, Germany). Feed samples were incubated in 60 mL of buffered rumen fluid and placed in a water bath at 39°C with continuous agitation for 48 h. The in vitro gas production experiment was conducted using a fully automated gas production technique described by Cone et al. (1996), in which the total gas volume is automatically recorded at 0.2-h intervals and corrected for the normal atmospheric pressure (101.3 kPa).

Gas Sampling

To predict CH_4 production in vivo based on the data from the in vitro system, we used the method of Ramin and Huhtanen (2012). Gas sampling was performed at 2, 4, 8, 24, 32, and 48 h of incubation by drawing a gas sample from each bottle in a gas-tight syringe (Hamilton, Bonaduz, Switzerland) through the rubber suba-

seal. A sample size of 0.2 mL of gas was injected into a gas chromatograph (Varian Star 3400 CX FID Gas Chromatograph; Varian Inc., Palo Alto, CA), equipped with a thermal conductivity detector. A standard mixture of CO₂ (900 mmol/mol) and CH₄ (100 mmol/mol) was used as a calibration gas (AGA Gas AB, Sundbyberg, Sweden), and gas sample peaks were recognized by comparison with the standard gas. The duration of the gas sampling process was approximately the same as that of the incubation process (35–45 min), ensuring an equal fermentation time in all bottles.

VFA, pH Measurements, and Digestibility Analysis

Each bottle was sampled for VFA analysis at 48 h of incubation by drawing a sample of 0.3 mL fluid residue using a liquid syringe. The fluid samples were pooled within treatment and run and stored in Eppendorf tubes at –18°C until further analysis. Concentration of VFA was determined by liquid chromatographic analysis using a Waters Acquity ultra-performance liquid chromatography apparatus (Waters, Milford, MA), as described in detail by Puhakka et al. (2016). The concentration of total VFA (mmol/L) was calculated as the difference between total VFA concentration in the sample and average total VFA concentration in the blanks. Total VFA production (mmol) was calculated by multiplying the difference in VFA concentration (sample – blank) by the sample volume (60 mL). The pH of each bottle at 48 h of incubation was also determined (744 pH Meter; Metrohm Ltd.). The *in vitro* digestibility was determined as true DM digestibility (TDMD). Sampling for TDMD was performed at 48 h of incubation by transferring the feed sample residue from each bottle into a preweighed polyester bag with a pore size of 11 µm. All excess liquid was carefully pressed out through the pores of the bag. The nylon bags were sealed and stored at –18°C until further analysis. On the day of analysis, the sample bags were thawed and boiled in an NDF solution for 1 h with added heat-stable α-amylase and sodium sulfite. Sample bags were dried at 60°C for 48 h before weighing.

Chemical Analysis

Dry matter concentration was determined by drying the grain (including the hull) and silage samples at 105°C for 16 h. Ash concentration was determined by incinerating the samples at 500°C for 4 h (AOAC International, 2012) and OM concentration was calculated as the difference between 1,000 and the ash concentration. Nitrogen concentration was determined using the Kjeldahl method (AOAC International, 2012) and a

Tecator digestion system (Tecator Digestion Auto and Tecator Scrubber; Foss Analytical, Hillerød, Denmark) and a distillation and titration apparatus (Kjeltec Auto 2300; Foss Analytical). We calculated CP concentration by multiplying the N concentration by 6.25. We determined crude fat concentration by ether extraction and HCl hydrolysis (Foss Soxtec 8000 extraction unit, SoxCap 2047 hydrolysis unit; Foss Analytical). The NDF concentration was determined using the method described by Van Soest et al. (1991), using a 1020 hot and 1021 cold extractor (Tecator Fibertec System; Foss Analytical) with addition of heat-stable α-amylase and sodium sulfite. The NDF concentration was expressed free of residual ash (**aNDF**). Indigestible NDF (**iNDF**) concentration was determined by applying a 288-h *in situ* incubation as described by Krizsan et al. (2015), using polyester bags with a pore size of 6 µm and a pore area equal to 5% of the total surface area. Sample bags were incubated in 2 lactating Ayrshire dairy cows fitted with a rumen fistula at the research farm at the Department of Agricultural Sciences at the University of Helsinki, Finland. The cows were fed a diet based on grass silage and concentrate (60:40 on a DM basis). Indigestible NDF was expressed inclusive of residual ash. Frozen silage samples were thawed and pressed before analysis. Ammonia-N was analyzed by direct distillation after adding MgO using a Kjeltec 2100 Distillation Unit (Foss Analytical). We analyzed VFA and lactic acid according to the method of Ericson and André (2010).

Predicted *In Vivo* Methane Production

We calculated the cumulative CH₄ production (mL) at each time point (0.2 h) according to the following equation:

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

where V_{CH_4} was the total CH₄ production at each time point; V_{HS} was the headspace volume; CH_4 was the CH₄ concentration in the headspace; V_{GP} was the gas production volume; and coefficient A was the ratio of CH₄ concentration in the outflow gas to the headspace. Coefficient A (0.55) was predicted using a mechanistic model described by Ramin and Huhtanen (2012).

Methane concentration at 0.2-h time intervals was estimated by fitting a logarithmic regression of measured CH₄ at 6 time points, as described by Ramin and Huhtanen (2012). Methane production (mL) at each time point (0.2) was calculated as total gas production × calculated CH₄ concentration. The kinetic param-

eters of total gas production and CH₄ production at each time point (0.2) were predicted separately by fitting the data to the 2-pool Gompertz model described by Schofield et al. (1994), using the NLIN procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC) according to the following equation:

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

where V_t is measured total gas or CH₄ volume at time t ; V_1 , k_1 , and L_1 are asymptotic cumulative gas production (mL/g of DM), rate (1/h), and lag (h) parameters, respectively, for the first pool (rapid); V_2 , k_2 , and L_2 are the corresponding parameters for the second pool (slow); and t is incubation time.

The parameters were subjected to a dynamic, mechanistic 2-compartment rumen model as described by Huhtanen et al. (2008), with modifications as described by Ramin and Huhtanen (2012). The model was used to predict the proportion of asymptotic CH₄ production at infinitive time that would be produced during the residence of feed in the rumen and the simulations were performed using POWERSIM version 2.5 (Powersim Software, Bergen, Norway) with a retention time of 50 h, which represented the maintenance level of intake. Predicted in vivo CH₄ production (mL/g of DM) was calculated as CH₄ = proportion $\times$ asymptotic CH₄ production (mL/g of DM) and expressed it as grams per kilogram of DM, calculated as follows:

$$\text{CH}_4 \text{ (g/kg of DM)} = \text{CH}_4 \text{ (mL/g of DM)} / \\ 22.4 \text{ (L/mol)} \times 16.04 \text{ (g/mol)},$$

where 22.4 is the gas volume and 16.04 is the molar mass of CH₄.

Stoichiometrically Predicted Methane Production

Stoichiometrically predicted CH₄ (**CH₄VFA**) production was calculated using the amounts of acetate, propionate, and butyrate produced in vitro according to the following equation (Wolin, 1960):

$$\text{CH}_4\text{VFA (mL)} = 22.4 \times (0.50 \times \text{AA} - 0.25 \\ \times \text{PA} + 0.50 \times \text{BA}),$$

where AA, PA, and BA are the total amounts (mmol) of acetate, propionate, and butyrate, respectively, in the feed samples at 48 h of incubation and 22.4 is gas volume (L/mol of gas). The VFA data were corrected for blanks.

Predicted Methane Production Using Mechanistic and Empirical Equations

The Nordic dairy cow model Karoline is a mechanistic dairy cow model described by Danfær et al. (2006). In this study, we used the sub-model predicting CH₄ production, recently revised by Huhtanen et al. (2015), to predict methane. The Karoline model requires a set of input variables, such as the chemical composition parameters of the feedstuff and the kinetic digestion rates for specific feed fractions. If required input variables were not analyzed, they were estimated or derived from feed tables. For grains and silage, potentially digestible NDF was calculated as the difference between aNDF and iNDF. The sugar concentration of oats (18 g/kg of DM) and barley (20 g/kg of DM) was derived from national feed tables (LUKE, 2019). Starch concentration for each individual grain variety was calculated as OM – CP – crude fat – aNDF – sugar. The grain protein fractions of amino nitrogen, peptide nitrogen, soluble protein nitrogen, insoluble protein, and indigestible protein for grains were derived from the Cornell Net Carbohydrate and Protein System feed table (Tylutki et al., 2008). Values for the digestion rate of NDF were set at 0.050 and 0.066/h for oats and barley, respectively. For the digestion rate of starch, we used a value of 0.2/h for both grains. The digestion rate of insoluble protein was set at 0.09 and 0.11/h for oats and barley, respectively. Silage protein fractions and digestion rates were based on the Cornell Net Carbohydrate and Protein System feed table (Tylutki et al., 2008). The Karoline simulations were based on a 60-kg sheep fed at maintenance level of intake (1 kg of DMI/d).

We also predicted CH₄ production using an empirical equation developed and described by Ramin and Huhtanen (2013), which is based on feed intake and the chemical composition of the feed, as follows:

$$\text{CH}_4 \text{ (L/kg of DMI)} = 5 - 0.35 \times \text{DMIBW} + 0.031 \\ \times \text{OMD}_m - 0.043 \times \text{EE} + 0.018 \times \text{NDF} \\ + 0.018 \times \text{NFC},$$

where DMIBW is DMI per unit of BW (g/kg); OMD_m is OM digestibility at a maintenance level of feed intake (g/kg); EE is ether extract (g/kg of DM); NDF is given in grams per kilogram of DM; and NFC (g/kg of DM) was calculated as OM – CP – NDF – crude fat (NRC, 2001). Empirical CH₄ production (g/kg of DM) was calculated as

$$\text{CH}_4 \text{ (g/kg of DM)} = \text{CH}_4 \text{ (L/kg of DMI)} \\ \times 1 \text{ (L)} / 22.4 \text{ (L/mol)} \times 16.04 \text{ (g/mol)},$$

where 22.4 is the gas volume and 16.04 is the molar mass of CH₄.

Statistical Analysis

All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc.). Data for total gas and CH₄ production parameters (g/kg of DM), TDMD (g/kg of DM), and pH values were subjected to ANOVA using the MIXED procedure in SAS according to the following model:

$$Y_{ijkl} = \mu + G_i + V(G)_{ij} + R_k + b_l + \varepsilon_{ijkl},$$

where Y_{ijkl} is the observation; μ is the overall mean; G_i is the effect of grain ($i = 2$); $V(G)_{ij}$ is the effect of variety within grain ($j = 16$); R_k is the effect of the run ($k = 3$); b_l is the random effect of bottle (position in bath); and ε_{ijkl} is the random error term. Because the samples for CH₄VFA (g/kg of DM), total VFA production (mmol), and molar proportions of VFA (mmol/mol) for each run were pooled within treatment, these data were subjected to ANOVA by a different model using the MIXED procedure in SAS:

$$Y_{ijk} = \mu + G_i + V(G)_{ij} + R_k + \varepsilon_{ijk},$$

where Y_{ijk} is the observation; μ is the overall mean; G_i is the effect of grain; $V(G)_{ij}$ is the effect of variety within grain; R_k is the random effect of the run; and ε_{ijk} is the random error term. We tested the data used in both models using the Shapiro-Wilk test for normality and homoscedasticity of residuals. The results were considered statistically significant when $P < 0.05$, and highly significant when $P < 0.01$. We adjusted P -values for multiple comparisons of variety within grain using Tukey's test. To investigate possible relationships between predicted in vivo CH₄ and grain composition (TDMD, OM, CP, aNDF, iNDF, and crude fat) a regression analysis was performed by using the REG procedure in SAS. To investigate possible interactions between grain species and grain composition on predicted in vivo CH₄ production, we performed a regression analysis using the GLM procedure in SAS.

RESULTS

Chemical Composition of Feeds

The chemical composition of the oat and barley varieties and the means and standard deviations within grain species are presented in Table 1. The most pro-

nounced differences between the oat and barley varieties were higher concentration of aNDF, iNDF, and crude fat in the oat varieties compared with the barley varieties. The silage had a DM concentration of 285 g/kg of fresh weight. The concentrations of OM, CP, aNDF, and iNDF were 938, 157, 611, and 102 g/kg of DM, respectively. The fermentation quality of the silage was good, in that it had low concentrations of ammonia N (45.0 g/kg of N). Concentrations of lactic acid, acetic acid, propionic acid, and butyric acid were 99.6, 21.6, 1.06, and 0.27 g/kg of DM, respectively.

Total Gas and Methane Production

The findings for total gas and predicted in vivo CH₄ production measurements are presented in Table 2. Total gas production and predicted in vivo CH₄ production were 8.2 and 8.9% lower ($P < 0.01$), respectively, in oat-based diets than in barley-based diets. The variety of grain species did not affect total gas or predicted in vivo CH₄ production. The mechanistic and empirical CH₄ predictions are illustrated in Figure 1. Both models expressed a good relationship to predicted in vivo CH₄; root mean square errors were 0.80 and 0.78 for the mechanistic and empirical models, respectively. Although R^2 values were not very high (0.67 and 0.68 for the mechanistic and empirical models, respectively), relative prediction errors were 3.6 and 3.5%, respectively, of the observed mean.

Relationships Between Predicted In Vivo Methane Production and Grain Composition Parameters

The results from simple and multiple regression analysis of predicted in vivo CH₄ production and grain parameters are presented in Table 3. The best predictor of CH₄ production was TDMD. Among grain composition parameters, iNDF concentration was the best predictor of CH₄ production. The mean response in CH₄ production to increased iNDF concentration was -0.020 g/kg of DM per 1 g/kg of DM in iNDF. Grain aNDF concentration was only slightly less associated with CH₄ production than iNDF concentration. Using crude fat concentration as a predictor instead of iNDF or aNDF increased the prediction error slightly. However, the mean response in CH₄ production to crude fat (-0.087 g/kg of DM per 1 g/kg of DM crude fat) was greater than that to iNDF or aNDF. Grain CP concentration did not affect CH₄ production. When the data were classified according to grain species ($n = 8$), we found no interactions between grain type and grain parameters ($P > 0.54$) with respect to predicted in

Table 1. Chemical composition of oat and barley varieties incubated in the in vitro gas production experiment

Grain	Variety	DM, g/kg	Composition, g/kg of DM ¹					NFC ²
			OM	CP	aNDF	iNDF	Crude fat	
Oats	Riina	911	970	132	311	144	53.5	474
	Nike	902	971	112	347	128	37.1	475
	Niklas	910	969	136	304	143	48.9	480
	Kerstin	908	974	124	391	158	41.7	417
	Meeri	912	970	139	308	147	52.9	470
	Akseli	911	968	134	320	148	60.9	453
	Cilla	910	969	123	369	150	49.6	427
	Haga	917	976	94	310	135	45.0	527
	Mean	910	971	124	332	144	48.7	466
	SD	3.9	2.8	14.9	32.7	9.4	7.40	34.0
Barley	6-row Justus	894	976	152	204	43.8	26.8	593
	6-row Kaarle	898	978	115	190	27.2	25.7	647
	2-row RGT Planet	897	982	105	170	42.9	29.1	678
	2-row Wootan	894	978	144	201	52.2	26.6	606
	6-row Elmeri	897	979	111	214	49.4	29.0	625
	6-row Severi	895	978	137	221	35.9	29.3	591
	2-row Selena	895	979	101	187	38.8	26.3	665
	2-row Tamtam	894	980	91	185	40.5	30.1	674
	Mean	896	979	119	197	41.3	27.9	635
	SD	1.6	1.8	22.2	16.7	7.80	1.70	35.9
Silage ³		285	938	157	611	102	—	—

¹aNDF = NDF free of residual ash; iNDF = indigestible NDF.

²NFC not analyzed; calculated as OM – CP – aNDF – crude fat (NRC, 2001).

³Concentration of ammonia N was 45.0 g/kg of N, lactic acid 99.6 g/kg of DM, acetic acid 21.6 g/kg of DM, propionic acid 1.06 g/kg of DM, and butyric acid 0.27 g/kg of DM.

vivo CH₄ production. Bivariate models did not improve predictions because of collinearity between variables (models not shown).

Digestibility and Fermentation Pattern In Vitro

Results for digestibility expressed as TDMD, pH at 48 h of incubation, and VFA production parameters are presented in Table 4. True DMD decreased by 7.0% in oat-based diets compared with barley-based diets ($P < 0.01$) and variety of grain species also affected TDMD ($P = 0.04$). We observed a negative relationship

between iNDF concentration and TDMD among the varieties of oats and barley (Figure 2). Oat-based diets increased the pH value at 48 h of incubation compared with barley-based diets ($P < 0.01$), whereas barley-based diets expressed a higher production of total VFA than oat-based diets ($P < 0.01$). In addition, variety of grain affected pH at 48 h of incubation ($P < 0.01$), but when P -values were adjusted for multiple testing, we observed differences only between some of the barley varieties, ranging from 0.06 to 0.10 pH units. Grain and variety of grain did not affect the molar proportions of VFA, except for a significantly greater proportion of

Table 2. Effects of grain species and variety within grain species on predicted in vivo total gas and methane production

Item	Oats	Barley	SEM		<i>P</i> -value	
			Oats	Barley	Grain	Variety
Total gas, g/kg of DM						
Asymptotic gas	160	173	3.4	3.2	<0.01	0.23
Predicted gas ¹	145	158	2.9	2.7	<0.01	0.30
CH ₄						
Asymptotic CH ₄ , g/kg of DM	25.6	28.2	0.41	0.39	<0.01	0.21
Rate, 1/h	0.063	0.060	0.0011	0.0011	0.03	0.16
Predicted in vivo CH ₄ , g/kg of DM	21.4	23.5	0.30	0.29	<0.01	0.17
Predicted in vivo CH ₄ , g/kg of TDMD ²	27.0	27.5	0.37	0.35	0.26	0.36
CH ₄ /total gas, % ³	14.9	15.1	0.22	0.21	0.50	0.67

¹Predicted in vivo total gas and CH₄ production based on observed values corrected for a mean retention time of 50 h.

²TDMD = true DM digestibility determined at 48 h of incubation in vitro.

³Predicted in vivo CH₄ (g/kg of DM) divided by predicted total gas (g/kg of DM).

Table 3. Relationships between grain variables (g/kg of DM) and predicted in vivo CH₄ production (g/kg of DM; n = 16)

Item ¹	Intercept	SE	P-value	Slope	SE	P-value	RMSE ²	Adjusted R ²
TDMD	−6.9	4.97	0.19	0.036	0.006	<0.01	0.74	0.694
aNDF	26.2	0.81	<0.01	−0.014	0.003	<0.01	0.85	0.589
iNDF	24.3	0.43	<0.01	−0.020	0.004	<0.01	0.84	0.602
Crude fat	25.8	0.75	<0.01	−0.087	0.019	<0.01	0.87	0.574

¹aNDF = NDF free of residual ash; iNDF = indigestible NDF; TDMD = true DM digestibility.

²RMSE = root mean square error.

valerate in barley-based diets compared with oat-based diets.

DISCUSSION

Chemical Composition of Feeds

Oat grain displays a greater proportion of hull than barley grain, accounting for approximately 25% in oats and 13% in barley, whereas barley displays a greater proportion of endosperm than oats, accounting for approximately 76% in barley and 63% in oats (Evers and Millar, 2002). The greater proportion of fibrous hull in oats is reflected in the higher aNDF concentration compared with barley. The greatest difference in aNDF concentration observed in this study was 221 g/kg of

DM between the oat variety Kerstin and the barley variety RGT Planet. The endosperm serves as a storage compartment for most of the starch in cereal grains, and the difference in endosperm proportion is reflected in the greater concentration of starch in barley than in oats (Evers and Millar, 2002). We did not analyze starch concentration in this study, but the calculated values (OM − CP − crude fat − aNDF − sugar) of 630 and 450 g/kg of DM in barley and oats, respectively, were within the normal ranges of 510 to 640 g/kg of DM (barley) and 400 to 500 g/kg of DM (oats) reported by Holtekjølen et al. (2006) and Sayer and White (2011).

Oats are known to have a higher oil concentration than barley; most of the oil (96%) is found in the groat, and the rest is found in the hull (Bryngelsson et al., 2002). The groat oil is largely found in the endosperm

Figure 1. The relationship between predicted in vivo CH₄ production and CH₄ production predicted by the mechanistic Nordic dairy cow model Karoline and the empirical equation developed by Ramin and Huhtanen (2013). RMSE = root mean square error.

Table 4. Effects of grain species and variety within grain species on in vitro TDMD, pH, total VFA production, VFA molar proportions, and stoichiometrically predicted CH₄ production at 48 h of incubation in vitro

Item	Oats	Barley	SEM		P-value	
			Oats	Barley	Grain	Variety
TDMD, ¹ g/kg of DM	795	855	1.9	1.8	<0.01	0.04
pH	6.26	6.11	0.005	0.005	<0.01	<0.01
Total VFA production, mmol/g of DM	3.30	3.68	0.11	0.11	<0.01	0.55
VFA molar proportions, mmol/mol						
Acetate	630	628	4.4	4.4	0.65	0.51
Propionate	216	214	1.8	1.8	0.63	0.27
Butyrate	130	129	5.0	5.0	0.95	0.88
Isobutyrate	3.9	5.0	0.49	0.49	0.12	0.59
Valerate	12.4	14.3	0.74	0.74	0.03	0.34
Isovalerate	1.1	2.7	0.57	0.57	0.08	0.56
CH ₄ VFA, ² mL	24.6	27.3	0.79	0.79	<0.01	0.44

¹TDMD = true DM digestibility determined at 48 h of incubation in vitro.

²CH₄VFA = stoichiometrically predicted CH₄ production using the amount of acetate, propionate, and butyrate produced in vitro (Wolin, 1960).

(86–90%), and smaller proportions are found in the scutellum and embryo (Banaś et al., 2007). Compared with the reported total variation in oil concentration among hulled oat varieties (30 to 110 g/kg of DM; Zhou et al., 1999), the varieties evaluated in this study were in the lower range (37–61 g/kg of DM); high-oil oat varieties in the upper range were not represented. The variations in crude fat concentration among the barley varieties were smaller (26–30 g/kg of DM) than among the oat varieties, but well in line with the reported variations (19–41 g/kg of DM; Welch, 1978).

The variations in CP concentration among the barley varieties found in this study (91–152 g/kg of DM) were well in line with reported variations (99–187 g/kg of DM; Welch, 1978). However, the variations in CP concentration among the oat varieties were smaller than among barley varieties (94–139 g/kg of DM) and compared with reported variations (112–160 g/kg of DM; Welch and McConnell, 2001).

Method

It is difficult to create an in vitro environment exactly similar to the in vivo environment, and our in vitro gas production system had some limitations. There is no absorption of VFA and no continuous intake of feed-stuff during incubation. However, the prediction error of CH₄ production was relatively small (9.5% of observed mean) in the study by Danielsson et al. (2017), in which predicted in vivo CH₄ values obtained from the in vitro gas production system were compared with observed values from in vivo studies that measured CH₄ using respiration chambers or the GreenFeed system (C-Lock Inc., Rapid City, SD). The estimated proportion of gross energy (Volden, 2011) lost as CH₄ ranged between 6.1 and 7.4%, close to the predicted values of

Ramin and Huhtanen (2012) at a maintenance level of feed intake using the same in vitro gas production approach as used in the present study. In addition, these values were also well in line with values obtained in vivo on grass silage-based diets (Yan et al., 2000).

Because the proportion of grain was only 50% (on a DM basis) of the incubated feed mixture in this study, the observed decrease in TDMD of 7 percentage units for the oat-based diets compared with the barley-based diets fell well in line with the values in national feed tables, which reported a 10- to 12-percentage-unit difference in digestibility between oats and barley (LUKE, 2019). In addition, the pH levels observed in this experiment (6.11 and 6.26 for barley and oats, respectively) at 48 h of incubation may be regarded as in the normal range, indicating that the buffered mineral solution used in vitro was able to imitate the effect of saliva excreted in vivo. Contrary to the findings of this study, Vanhatalo et al. (2006) reported no differences in pH between oats and barley when compared in vivo on a grass-silage-based diet. This inconsistency may be explained by the differences between the in vitro and in vivo environments. The variation in pH at 48 h of incubation expressed a close relationship ($R^2 = 0.90$) with the variation in TDMD among the different grain varieties, whereas total VFA production was less associated ($R^2 = 0.38$) with the variation in pH.

The observed decrease in total VFA production for oat-based diets compared with barley-based diets was consistent with the results of Vanhatalo et al. (2006) and can be explained by the lower digestibility of oats compared with barley. However, the decrease in total VFA production was 10%, whereas the decrease in TDMD was only 7%, indicating that additional factors lowered total VFA production. Because dietary fatty acids are not fermented in the rumen and do not con-

Figure 2. The relationship between true DM digestibility (TDMD) determined in vitro and indigestible NDF (iNDF) concentration of 16 varieties of oats and barley incubated in vitro over 48 h. Observations grouped to the left represent barley varieties, and observations grouped to the right represent oat varieties. RMSE = root mean square error.

tribute to the VFA pool, the higher fat concentration of oats may be an additional factor explaining the lower total VFA production observed for oat-based diets.

Total Gas and Methane Production

To our knowledge, no study has compared CH_4 production between oat- and barley-based diets or between different varieties of the grains in question. The decrease in total gas and predicted in vivo CH_4 production we observed for oat- compared with barley-based diets was most likely related to decreased digestibility, because grain species did not affect CH_4 production relative to TDMD. In the present study, the silage and percentage of grain inclusion were the same for all treatments, and the differences in total gas and CH_4 production between oat- and barley-based diets were most likely related to differences in chemical composition between the grains.

The strong positive relationship ($R^2 = 0.69$) observed between diet TDMD and CH_4 production in the present study was also confirmed by other studies (Blaxter and Clapperton, 1965; Ramin and Huhtanen, 2013), and by the fact that CH_4 production arises only from the fermentation of digestible matter in the rumen. A better fit between digestible crude nutrients and CH_4 production compared with using crude nutrients in the equation was reported by Jentsch et al. (2007). The negative relationship between crude fat concentration in the grains and CH_4 production we observed in the

present study confirmed the findings of several earlier studies (Czerkawski et al., 1966; Beauchemin et al., 2009; Grainger and Beauchemin, 2011). In the present study, CH_4 production decreased by 0.087 g/kg of DM when dietary crude fat concentration increased by 1 g/kg of DM. However, this response in CH_4 production was also influenced by the lower digestibility of oats; as a result, a smaller response in CH_4 production to increased dietary fat concentration would be expected. Ramin and Huhtanen (2013) reported a 0.043 L/kg of DM decrease (equal to 0.031 g/kg of DM) in CH_4 production when ether extract was used in the equation and a 0.096 L/kg of DM decrease (equal to 0.069 g/kg of DM) when ether extract was replaced by fatty acids in the same study.

The CH_4 -mitigating effect of increased dietary fat concentration may be explained by 3 underlying mechanisms. First, fermentable substrate is replaced with non-fermentable fatty acids (Johnson and Johnson, 1995), decreasing the extent of fermentation and leading to a lower amount of H_2 available for methanogenesis. Second, dietary fat suppresses the function of fiber-digesting microbes in the rumen, inducing a shift in fermentation pattern toward propionate production at the expense of acetate and butyrate (McAllister et al., 1996). The propionate-producing pathway captures H_2 and acts as an H_2 sink in the rumen. However, this was not the case in the present study, because the fermentation pattern was not affected by grain spe-

cies. In comparison, Vanhatalo et al. (2006) reported a slightly lower proportion of butyrate when barley was replaced by oats in an *in vivo* study on a grass-silage-based diet. Third, the biohydrogenation of dietary UFA captures H_2 and thus acts as another H_2 sink in the rumen (Czerkawski et al., 1966). However, the biohydrogenation pathway plays only a minor role in the CH_4 -mitigating effect of increased dietary fat, because the complete biohydrogenation of 1 mol of linoleic acid would decrease CH_4 production by only 1 mol. Assuming that the fatty acids in oats contain 1.5 double bonds, complete biohydrogenation could decrease CH_4 by approximately 0.4 g/kg of DM. Therefore, the CH_4 -mitigating mechanism of increased crude fat concentration in oats is mostly due to the replacement of fermentable substrate by nonfermentable substrate (fatty acids) in the diet, an explanation that is also supported by the decrease in total VFA production in oat- compared with barley-based diets.

In this study, the nonsignificant effects of iNDF and crude fat on CH_4 production when they were included in the same model is related to the high correlation between these composition parameters, indicated by a high variance inflation factor (5.9). In addition, the variance inflation factor was relatively high (4.0) between TDMD and crude fat concentration, resulting in a nonsignificant effect of crude fat when included with TDMD in the bivariate model. Due to the collinearity problem between grain composition parameters and digestibility, it was difficult to draw any further conclusions about the relative importance of the CH_4 -mitigating mechanisms of oats from this study.

Although oats and barley have additional differences in chemical composition, such as the concentrations of soluble β -glucans, phenolic compounds, and avenanthramides, these factors did not seem to play a role in the CH_4 -mitigating effect of oats, because the decrease in CH_4 production when replacing barley with oats was as expected based on differences between the grains in terms of digestibility and oil concentration. In addition, the difference between oats and barley in predicted CH_4 VFA values (10%) was close to the observed difference (9.8%) when the CH_4 VFA was compared with observed CH_4 endpoint values at 48 h of incubation. Because the CH_4 VFA equation by Wolin (1960) is based on the amount of digestible carbohydrates to predict CH_4 production through major VFA molar proportions, the CH_4 -mitigating effect of oats was accounted for by the decreased digestibility and replacement of fermentable carbohydrates by fat. The mechanistic and empirical CH_4 values expressed close relationships to predicted *in vivo* CH_4 production values. The relationships were similar between mechanistic CH_4 predictions

and predicted *in vivo* CH_4 , as well as between empirical CH_4 predictions and predicted *in vivo* CH_4 . In contrast to our second hypothesis, grain variety did not significantly affect CH_4 production, because of relatively small variations in digestibility and oil concentration between different varieties of the 2 grains.

CONCLUSIONS

Production of CH_4 was 8.9% lower for oat-based diets than for barley-based diets, and *in vitro* TDMD and total VFA production were also lower for oat-based diets than barley-based diets. The CH_4 -mitigating effect of oats observed in the present study was most likely related to the lower digestibility and higher oil concentration of oats compared with barley. Based on the results of this experiment, we conclude that replacing barley with oats in a grass-silage-based diet could decrease enteric CH_4 production in dairy cows. Differences between grain varieties did not reach statistical significance. The relationships between composition and CH_4 production suggest that selecting grain varieties with low iNDF and high fat concentration can reduce CH_4 emissions.

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