Animal Feed Science and Technology 184 (2013) 67–79

Contents lists available at SciVerse ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Enteric methane production, digestibility and rumen fermentation in dairy cows fed different forages with and without rapeseed fat supplementation

M. Brask*, P. Lund, A.L.F. Hellwing, M. Poulsen, M.R. Weisbjerg

Aarhus University, Department of Animal Science, AU Foulum, PO Box 50, DK 8830 Tjele, Denmark

ARTICLE INFO

Article history:
Received 18 February 2013
Received in revised form 13 June 2013
Accepted 15 June 2013

Keywords:
Canola
Cattle
Fiber digestibility
Grass silage
Maize silage

ABSTRACT

The purpose of this experiment was to study the effect of forage species (grass or maize) and the maturity stage of grass on enteric methane (CH_4) production, nutrient digestibility and rumen fermentation, and to study possible interactions with cracked rapeseed as fat source. Six lactating, ruminal, duodenal and ileal cannulated Holstein dairy cows (206 days in milk, milk yield 25.1 kg) were submitted to an incomplete Latin square design (6×4) with six diets and four periods. Two grass silages (early first cut, 361 g aNDFom/kg DM and late first cut, 515 g aNDFom/kg DM) and one maize silage were supplemented with either low fat concentrate or high fat concentrate. The dietary fat concentration in the high fat diets was approximately 60 g/kg DM. Diurnal samples of duodenal and ileal digesta and feces were compiled. The CH_4 production was measured for four days in open-circuit respiration chambers. Additional fat reduced the gross energy (GE) lost as CH_4 from 6.3 to 5.8% of GE intake, independent of forage species and quality. Energy loss as CH_4 constituted 6.1, 6.7 and 5.4% of GE intake for early grass silage, late grass silage and maize silage, respectively. However, there was no difference between early grass silage and maize silage when CH_4 production was related to kg organic matter (OM) digested. Fat supplementation did not affect OM or aNDFom digestibility. Maize silage had a higher ruminal OM digestibility, but lower ruminal aNDFom digestibility than grass silage. Early cut grass silage had a higher total tract OM and aNDFom digestibility than late cut grass silage. The present study demonstrates that choice of forage species and harvest time affects CH_4 emission from dairy cows, while the CH_4 reducing ability of fat does not interact with forage characteristics.

1. Introduction

Enteric CH_4 production is influenced by the chemical composition of the diet. Fat supplementation has been studied intensively and found to be a very promising tool to decrease CH_4 from rumen fermentation since fat is not fermented to any great extent in the rumen and certain fat sources such as unsaturated fat might additionally affect methanogenic fermentation pathways (Beauchemin et al., 2008). Modeling studies (Jentsch et al., 2007; Ramin and Huhtanen, 2013) suggest that CH_4

Abbreviations: aNDFom, NDF assayed with a heat stable amylase and expressed exclusive of residual ash; BW, body weight; CH_4, methane; CP, crude protein; DM, dry matter; FA, fatty acid; GE, gross energy; NH_3, ammonia; OM, organic matter; TMR, total mixed ration; VFA, volatile fatty acids; DMI, dry matter intake; ECM, energy corrected milk; EG, early harvest primary growth grass-clover; MS, maize silage; LG, late harvest primary growth grass-clover; INDF, indigestible NDF; GEI, GE intake; EGC, early grass silage control; EGF, early grass silage fat; LGC, late grass silage control; LGF, late grass silage fat; MSC, maize silage control; MSF, maize silage fat; SEM, standard error of mean; LSM, least square mean; DNDF, digestible NDF; NDF, neutral detergen fiber.

* Corresponding author. Tel.: +45 87154253; fax: +45 87154249.
E-mail address: Maike.Brask@agrsci.dk (M. Brask).

0377-8401/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.anifeedsci.2013.06.006

production also depends on other nutritional factors than fat, *e.g.* carbohydrate composition (starch, sugar and fiber) and diet digestibility. However, studies that at the same time have addressed the effect of forage species and quality on CH_4 production as well as rumen digestibility and rumen fermentation are scarce. Forage is an important part of a dairy cow diet and the forage carbohydrate composition plays an important role as high neutral detergent fiber (NDF) concentration in the forage can be expected to enhance acetic acid production in the rumen and thereby CH_4 production. Dietary starch, on the other hand, enhances propionic acid production and, according to stoichiometry, decreases CH_4 (Boadi et al., 2004). Dietary carbohydrate composition can vary considerably depending on forage species as whole-crop maize silage has a starch concentration around 30% while the starch concentration in grass or grass-clover silage is negligible. Furthermore, with increasing grass maturity, NDF concentration increases while NDF digestibility decreases (Rinne et al., 1997). It is well-known that starch concentration, NDF concentration and NDF digestibility influence rumen fermentation characteristics and passage rate (Rinne et al., 1997; Owens et al., 2009). Forage with a higher digestibility will lead to more intensive fermentation in the rumen (Rinne et al., 1997) and thereby increase in daily CH_4 production. On the other hand, increased forage digestibility results in increased passage rate and feed intake and decreased fiber intake compared with lower digestible forage, thereby reducing the amount of CH_4 per kg feed intake (Boadi and Wittenberg, 2002). However, it is not clear if the decreasing effect fat has on CH_4 production interacts with silage quality. Fat supplementation primarily may inhibit fibrolytic bacteria while other carbohydrates remain almost unaffected (Doreau and Chilliard, 1997). As the role of fibrolytic bacteria is more important in diets rich in fiber, it is expected that fat supplementation will affect CH_4 production more with increasing fiber concentration of the diet.

The aim of this paper was to study the methanogenic potential of three different silages and eventual interactions with rapeseed fat supplementation.

2. Materials and methods

2.1. Animals and diets

The experiment complied with the guidelines of the Danish Ministry of Justice with respect to animal experimentation and care of animals under study (approval number 2012-15-2934-00618).

Six lactating Danish Holstein dairy cows (4 primiparous and 2 multiparous) were assigned to one of six diets over four periods according to an incomplete Latin square design. Each period lasted for four weeks.

The cows were 206 days in milk (SD = 63 d) and had a milk yield of 25.1 kg (SD = 4.9 kg) and a BW of 550 kg (SD = 78 kg) at the beginning of the experiment. All animals were fitted with a ruminal cannula (#1C, Bar Diamond Inc., Parma, ID, USA), a duodenal cannula (open T-piece placed 60 cm caudal to pylorus) and an ileal cannula (open T-piece placed 20 cm cranial to caecum). The cows were housed in tie stalls with rubber mats and sawdust as bedding and had free access to water. They were milked and fed twice daily at 6:00 and 17:00. Total mixed rations were prepared once a day and fed to the cows on *ad libitum* basis after each milking. Refusals were kept at 2–3 kg in order to minimize selection. Feed and refusals were analyzed for DM. The feed intake was recorded on daily basis. The cows were weighed at the start of the experiment as well as just before and after the methane measurements in the last week of each period.

Three silages were used in the experiment: early harvest primary growth grass-clover (EG), late harvest primary growth grass-clover (LG) and maize silage (MS). The two grass-clover silages were harvested from the same field at AU Foulum (56.49 N 9.58 E) on May 26th and on June 15th, 2010, respectively. The sward consisted of perennial ryegrass, red clover and white clover; clover concentration was low (<10%) in both silages. All silage was stored in bales and ensiled without additive. The diets were fed as TMR with a forage to concentrate ratio of 65:35 on a DM basis. The concentrate components were from the same batch during the whole experiment and two concentrate pre-mixtures were prepared prior to each period and pelleted into pellets with 5 mm diameter. Low-fat concentrate included rapeseed meal, wheat, and mineral and vitamin supplement. The higher fat content in the high-fat concentrate was obtained by including whole cracked rapeseed. Other components were reduced proportionally to fat added and fat-free rapeseed concentration kept constant by reducing rapeseed meal according to the fat free rapeseed supplemented in rapeseed. Rapeseed was a double-00 variety obtained from Danraps (DLG Food Oil, Dronninglund, Denmark). Chemical composition and digestibility of feed components are shown in detail in Table 1. The three silages were each supplemented with either the low-fat concentrate (control, C) or high-fat concentrate (F) resulting in six different diets fed (EGC, EGF, LGC, LGF, MSC, MSF, Table 2). Two concentrates were used in order to achieve a better comparability of the diets. It was accepted that this resulted in differences in dietary N concentration. The diets were calculated so that the lowest N concentration supplied sufficient N according to NorFor recommendations (NorFor, 2013).

2.2. Intake, milk yield and digestibility measurements

The milk production and composition were measured once a week during morning and evening milkings. Weekly samples of the feed ingredients were stored (−20 °C) and pooled for the whole experiment. Samples of TMR and refusals were taken daily in connection with the morning feeding on days 15–19, stored (−20 °C) and pooled for each period.

Chromic oxide (Cr_2O_3) was used as a flow marker, and 10 g were administrated to the rumen via the ruminal cannula during each of the two daily feedings except when the cows were in the respiration chambers at the end of each period.

Table 1
Chemical composition (g/kg DM unless noted), energy concentration (MJ/kg DM) and digestibility of feed components.

	Feed component[a]							
	EG	LG	MS	Wheat	RSM	CR	C	F
DM (g/kg) fresh matter	422	329	306	893	924	942	913	914
OM	911	922	968	984	922	958	912	920
CP	168	124	93.0	115	380	194	294	264
Crude fat	36.9	24.8	31.7	23.3	46.9	453	41.0	118
Starch			150	650			123	117
Sugar	172	14.0	0.60					
aNDFom	361	515	437	129	248	181	210	188
INDF[b]	30.2	80.2	80.5	18.4	133	76.5		
Lignin	14.7	22.7	20.9					
GE[c] (MJ/kg)	18.0	17.9	18.7	17.9	19.3	28.2	18.3	20.1
OMD[d]	0.83	0.71	0.72	0.97	0.83	0.88	0.87	0.89
Fatty acids (g/kg DM)								
16:0	3.42	2.67	4.00	4.40	3.35	19.1		
18:0	0.37	0.29	0.39	0.18	0.66	6.27		
18:1n-9	0.64	0.85	5.08	3.30	21.7	242		
18:2n-6	2.99	2.42	10.8	12.3	10.5	76.4		
18:3n-3	15.5	8.09	2.75	1.03	2.93	39.8		
Total FA[e]	24.1	15.4	24.0	21.6	41.0	397		

[a] Feed components: EG, early grass silage; LG, late grass silage; MS, maize silage; RSM, rapeseed meal; CR, cracked rapeseed; C, concentrate pre-mixture control (low fat), F, concentrate pre-mixture high fat.
[b] INDF, indigestible NDF, residue after 288 h nylon bag incubation (12 μm pore size).
[c] GE, gross energy.
[d] OMD, *in vitro* organic matter digestibility.
[e] Sum of fatty acids includes beside FA shown also 16:1n-9, 20:0, 20:1n-9, 20:2n-6 and 22:0.

The first two weeks of each period (days 1–14) were used for adaptation. From days 15–19, twelve samples, representing every second hour of the day, of duodenal (600 mL) and ileal chyme (300 mL) and feces (350 mL) were taken at 10:00, 18:00 (day 15), 2:00, 12:00, 20:00 (day 16), 4:00, 14:00, 22:00 (day 17), 6:00, 16:00, 24:00 (day 18) and 8:00 (day 19). Samples from the duodenum and ileum were taken in tube-formed plastic bags which were mounted to the cannulas with plastic knees. Duodenal, ileal and fecal samples were at each sampling time added to the frozen pooled sample from previous samplings. At the end of each period, representative subsamples from thawed material were taken and freeze-dried for chemical analyses. At the 12 sampling times, rumen liquid was sampled from the ventral ruminal sac with a collection tube (#RT, Bar Diamond Inc., Parma, ID, USA). The rumen liquid pH was measured immediately, and two 8 mL samples were taken and frozen immediately (−20 °C) for VFA and ammonia (NH_3) analysis at each sampling time.

Table 2
Dietary ingredients and chemical composition of total mixed rations (g/kg DM unless noted).

	Diet[a]					
	EGC	EGF	LGC	LGF	MSC	MSF
Wheat, rolled	96	93	96	93	96	93
Rapeseed meal	241	195	241	195	241	195
Whole crushed rapeseed		72		72		72
Early grass silage	650	626				
Late grass silage			650	626		
Maize silage					650	626
Mineral mix	12	12	12	12	12	12
ADE vitamin	1	1	1	1	1	1
DM (g/kg fresh matter)	512	520	421	428	388	402
OM	908	915	922	924	948	949
aNDFom	304	299	407	391	355	337
CP	209	204	180	178	164	155
Crude fat	37.4	64.2	30.5	58.3	34.3	61.9
Fatty acids	29.0	53.5	23.1	47.5	28.8	55.6
Starch[b]	43.1	43.6	43.1	43.6	141	137
Sugar[b]	135	131	32.5	32.0	27.3	27.0
GE[c] (MJ/kg DM)	17.9	18.5	18.0	18.7	18.5	19.1

[a] Diets: EGC, early grass silage control; EGF, early grass silage fat; LGC, late grass silage control; LGF, late grass silage fat; MSC, maize silage control; MSF, maize silage fat.
[b] Calculated from ingredient analyses, table values were used for concentrate sugar concentration.
[c] GE, gross energy.

2.3. Methane measurements

During days 21–28 of each period, the CH_4 production was measured for each cow for 2 × 48 h in four 17 m^3 open-circuit respiration chambers (Hellwing et al., 2012). The chambers were covered with transparent polycarbonate and placed in a square so that the cows faced each other. The chambers were located in the barn where the cows were housed to minimize changes in the environment and the daily routines during the CH_4 measurements were identical to the rest of the feeding period. The mean ambient temperature in the chambers was 18.1 °C, ranging from 16.5 °C to 19.7 °C.

Four chambers were available and the cows were staggered in a way that two chambers were used on days 20 and 21, four from days 22–25 and two on days 26 and 27. After the first 48 h, the cows changed chambers diagonally in order to balance out any possible differences in background levels of CH_4. Cow and chamber were confounded over periods and therefore every ration was tested in every chamber.

The chambers were opened twice daily at 6:00 h and 17:00 h for about 20 min during milking and subsequent feeding. Methane was measured as the accumulated amount in L over 24 h and is reported under standard conditions (0 °C, 101.325 kPa). The measurements during the openings of the chambers for milking and feeding were deleted. The CH_4 production during this period was assumed to correspond to the mean of the rest of the day.

The air flow was measured with a mass flow meter from Teledyne Hastings Instruments (Hampton, Virginia, USA). The background (inlet air) as well as the chamber outlet air concentration of CH_4 was measured every 12½ min with an infrared analyzer. All instruments were obtained from Columbus Instruments, Columbus, OH, USA. The instruments were calibrated every second day with zero gas (nitrogen) and a span gas with nitrogen and 20.55% O_2, 5000 ppm CO_2 and 800 ppm CH_4 (Yara Praxair AS, Oslo, Norway). The recoveries of CO_2 and CH_4 in the chambers were 99.5% (SD = 2.6) within the experimental period. For further details see Hellwing et al. (2012).

2.4. Chemical analysis

The DM of feed samples was analyzed by drying at 60 °C for 48 h. Ash was determined by combustion at 525 °C for 6 h (method 923.03; AOAC, 1990). Nitrogen was determined by the Kjeldahl method (method 978.02; AOAC, 1990), and the CP was calculated as N × 6.25. Crude fat was extracted with petroleum ether (Soxtec 2050, Foss Analytical, Hillerød, Denmark) after hydrolyzing with HCl (Stoldt, 1952). The aNDFom concentration was analyzed by a neutral detergent extraction according to Mertens (2002) with a Fibertec™ M6 System (Foss Analytical, Hillerød, Denmark) using heat stable amylase and sodium sulfite and corrected for ash. The INDF content in freeze-dried ground (1.5 mm) samples was determined as residual NDF after 288 h (12 days) of dacron bag incubation with 12 μm pore size in the rumen of three heifers fed a standard ration at maintenance level (Åkerlind et al., 2011). The diet consisted of grass/clover hay, barley straw and a pelleted concentrate mixture consisting of 40 kg barley grain, 40 kg oat grain, 10 kg soybean meal, 3 kg rapeseed meal, 3 kg sugar beet molasses and 4 kg of a commercial mineral mixture (6 g per 100 g Ca, 10 g per 100 g P, 12 g per 100 g Mg, 5 g per 100 g Na; Type 3, Vitfoss, Gråsten, Denmark) per 100 kg fresh feed. The forage to concentrate ratio was 670:330 on DM basis and crude protein concentration of the diet was 139 g per kg DM. The forage consisted of one third barley straw and two thirds hay. The daily ration was divided into two meals of equal size. The OM digestibility was determined *in vitro* for grass and maize silage. Samples were analyzed in duplicate in separate runs. Samples were incubated in rumen fluid for 48 h followed by 48 h digestion in pepsin and HCl according to Tilley and Terry (1963). Residues were combusted to determine *in vitro* OM digestibility. Rumen fluid was collected from the 3 heifers also used for determination of INDF. *In vitro* OM digestibility of concentrates was determined as described by Weisbjerg and Hvelplund (1993). Samples were incubated in pepsin and HCl for 24 h and afterwards incubated in enzyme-acetatbuffer for further 24 h. Residues were combusted to determine *in vitro* OM digestibility. Lignin (sa) was determined according to ISO method 13908 (ISO, 2008). Starch was analyzed by an enzymatic calorimetric technique (Knudsen et al., 1987). Total sugar in grass silage was analyzed by the Luff-Schoorl method (European Community, 2012, 71/250/EEC). The GE was determined by an adiabatic bomb calorimeter (Parr 6300 Oxygen Bomb Calorimeter, Parr Instrument Company, Moline, IL, USA).

The concentrations of VFA were analyzed according to the method described by Canibe et al. (2007) using a Hewlett Packard gas chromatograph (model 6890) equipped with a flame ionization detector and a 30 m SGE BP1 column (Scientific Instrument Services, NJ, USA). Fatty acids in feed were analyzed by GC (HP 6890 series GC system) after an acidic Bligh & Dyer extraction and subsequent methylation and 17:0 as internal standard as described by Jensen (2008).

For determination of NH_3, the rumen fluid was made alkaline with KOH, and NH_3 was determined by titration after distillation using a Kjeltec 2400 (Foss Analytical, Hillerød, Denmark). Chromic oxide was determined by colorimetry after oxidation to chromate (Schürch et al., 1950). Milk concentrations of fat, protein and lactose were analyzed on a Milkoscan Msc4000 infrared analyzer (Foss Analytical, Hillerød, Denmark).

2.5. Calculations and statistical analysis

Chemical analyses on TMR samples were used for digestibility calculations. The apparent rumen digestibility was calculated as the feed intake minus the duodenal flow divided by feed intake for all nutrients except for aNDFom. For aNDFom rumen digestibility was also calculated using ileal flow instead of duodenal flow because calculations based on duodenal flow resulted in unreasonable results, indicating problems with either marker distribution or sampling method. It can be

assumed that no aNDFom digestion occurred in the small intestine. Apparent small intestine digestibility was calculated as the difference between duodenal and ileal flow divided by duodenal flow and apparent large intestine digestibility accordingly as the difference between ileal and fecal flow divided by ileal flow. Apparent total tract digestibility was calculated as the feed intake minus fecal flow divided by feed intake.

Energy corrected milk (3.14 MJ/kg) was calculated according to Sjaunja et al. (1991) as: ECM = milk yield × (383 × fat% + 242 × protein% + 783.2)/3140 using milk production and composition records from the last week of the period (during CH_4 measurements).

The data were evaluated with the MIXED procedure (SAS 9.2 version, SAS Institute Inc., Cary, NC) with period, silage (EG, LG and MS), concentrate (low or high fat) and a concentrate × silage interaction as fixed effects and cow as a random effect.

The results are reported as least square mean (LSM) and standard error of mean (SEM) for each treatment. P values < 0.05 were regarded as significant, P<0.10 as a tendency. Orthogonal contrasts were calculated for grass silages *vs.* maize silage (EG and LG *vs.* MS) and for comparing the two grass silages (EG *vs.* LG).

3. Results

3.1. Feed intake

Dry matter intake (DMI) was lower for LG than for EG and MS, whereas aNDFom intake was lowest for EG due to lower aNDFom concentration in the silage (Table 3). Organic matter intake was not affected significantly by fat supplementation but decreased numerically for EGF (0.5 kg) and MSF (1.0 kg) compared with low-fat diets. Crude protein intake was reduced in the fat supplemented diets due to the numerically lower DMI and CP content. Fatty acid intake and the proportion of 18:1n-9 and 18:2n-6 increased with rapeseed fat supplementation. Grass silage supplied more 18:3n-3 than maize silage; maize silage on the other hand supplied more 18:2n-6.

3.2. Digestibility

Ruminal OM digestibility was 3.6 percentage units higher for MS than for grass silage diets (P<0.001). Ruminal aNDFom digestibility on the other hand was 12.8 percentage units higher for the grass silage diets than for MS (P<0.001). Early grass and LG diets did not differ in OM or CP ruminal digestibility. Ruminal aNDFom digestibility did not differ between EG and LG when calculated based on ileal flow (P=0.12). Fat supplementation tended to decrease ruminal OM digestibility for EG and LG but not for MS. Rumen crude fat digestibility was negative for all diets and increased by fat supplementation.

Total tract digestibility of OM and aNDFom was higher on EG diets compared with LG and MS. There was no significant effect of fat supplementation on total tract digestibility of any nutrient. In the large intestine, aNDFom digestibility was high for EGF (0.23).

3.3. VFA, ammonia and rumen pH

Total VFA concentration was higher on EG than on LG and MS (P<0.001) but not affected by fat supplementation (P=0.35, Table 4). Replacing grass silage with maize silage increased propionic acid and decreased acetic acid proportion in the rumen (P<0.001) but there was no difference between EG and LG in molar propionic or acetic acid proportion. Consequently, acetate to propionate ratio was reduced (P<0.001) on MS (2.06) compared with EG and LG diets (2.63 and 2.68, respectively). Butyric acid proportion was lowest for EG (P<0.001) with no difference between LG and MS (P=0.65, contrasts not shown).

Rumen ammonia concentration was higher on EG and LG compared with MS (P=0.01). Fat supplementation numerically increased NH_3 concentration for EG and MS but not for LG. Rumen pH was on average 6.3 (average for 24 observations). Rumen pH was highest on LG. There was no difference in rumen pH between EG and MS (P=0.26). Fat supplementation tended to reduce rumen pH (P=0.06).

3.4. Milk production

Milk production was on average 21.8 kg/d and ECM production 23.8 kg/d. Neither milk production nor composition was affected by silage type or fat supplementation (Table 5). Fat supplementation numerically increased milk fat concentration and fat yield for EG and LG but not for MS.

3.5. Methane production

The cows produced on average 502 L CH_4/d varying from 455 L/d for cows on MSF to 542 L/d for cows on LGC. Fat addition decreased CH_4 as % of GE intake (GEI) from 6.31 to 5.83% (Table 6). Compared with EG and MS, LG resulted in more CH_4/kg DMI and per kg totally digested OM. There was less CH_4 produced on MS than EG when not taking digestion into consideration. However, there was less CH_4 per kg totally digested OM produced on EG than on MS. Feeding LG resulted in least CH_4 when related to rumen or totally digested aNDFom. The effects of silage type and fat supplementation were additive.

Table 3
The effect of silage type and rapeseed fat supplementation on intake, duodenal flow and apparent digestibility of nutrients.

	Diet[a]						SEM	P_{silage}	P_{fat}	$P_{silage*fat}$	Contrast P-values	
	EGC	EGF	LGC	LGF	MSC	MSF					EG and LG *vs.* MS	EG *vs.* LG
DM												
Intake (kg/d)	17.6	17.3	16.0	16.1	17.6	16.8	0.90	0.02	0.22	0.52	0.21	0.01
OM												
Intake (kg/d)	16.2	15.8	14.7	14.9	16.9	16.0	0.85	0.01	0.29	0.54	0.02	0.02
Duodenal flow (kg/d)	9.76	9.87	8.42	9.53	10.1	8.83	0.51	0.05	0.94	0.01	0.78	0.02
Rumen digestibility	0.398	0.376	0.424	0.356	0.405	0.444	0.019	0.01	0.10	0.004	0.004	0.77
Total tract digestibility	0.764	0.772	0.722	0.704	0.713	0.708	0.010	<0.001	0.45	0.33	0.001	<0.001
aNDFom												
Intake (kg/d)	5.41	5.15	6.43	6.31	6.36	5.69	0.35	0.002	0.09	0.49	0.33	<0.001
Duodenal flow (kg/d)	1.69	1.54	2.07	2.37	3.19	2.38	0.16	<0.001	0.10	0.01	<0.001	0.001
Rumen digestibility[b]	0.697	0.682	0.638	0.627	0.493	0.477	0.027	<0.001	0.54	0.99	<0.001	0.05
Rumen digestibility[c]	0.695	0.702	0.668	0.620	0.506	0.579	0.022	<0.001	0.57	0.06	<0.001	0.03
Hindgut digestibility	0.128	0.207	0.064	0.052	0.090	0.097	0.046	0.08	0.52	0.58	0.63	0.03
Total tract digestibility	0.737	0.753	0.662	0.646	0.543	0.531	0.017	<0.001	0.79	0.59	<0.001	<0.001
DNDF[d]												
Intake (kg/d)	4.47	4.24	5.07	4.97	4.82	4.28	0.29	0.02	0.11	0.57	0.44	0.01
Rumen digestibility	0.826	0.817	0.780	0.778	0.595	0.586	0.030	<0.001	0.79	0.99	<0.001	0.17
Total tract digestibility	0.875	0.881	0.821	0.816	0.665	0.672	0.019	<0.001	0.87	0.94	<0.001	0.008
Starch												
Intake (kg/d)	0.76	0.76	0.70	0.71	2.55	2.32	0.10	<0.001	0.28	0.26	<0.001	0.49
Duodenal flow (g/d)	230	225	152	128	226	225	34.2	0.02	0.70	0.94	0.14	0.01
Rumen digestibility	0.709	0.695	0.775	0.830	0.909	0.907	0.028	<0.001	0.58	0.45	<0.001	0.003
Digested in rumen (kg/d)	0.53	0.54	0.54	0.58	2.33	2.10	0.09	<0.001	0.32	0.21	<0.001	0.72
Sugar												
Intake (kg/d)	2.47	2.22	0.53	0.51	0.47	0.45	0.08	<0.001	0.14	0.26	<0.001	<0.001
Crude fat												
Intake (kg/d)	0.66	1.11	0.51	0.93	0.60	1.04	0.05	<0.001	<0.001	0.93	0.46	<0.001
Duodenal flow (kg/d)	1.03	1.53	0.73	1.19	0.85	1.24	0.06	<0.001	<0.001	0.36	0.05	<0.001
Small intestine digestibility	0.750	0.699	0.752	0.736	0.795	0.704	0.017	0.13	<0.001	0.04	0.16	0.13
Total tract digestibility	0.572	0.572	0.620	0.650	0.685	0.637	0.032	0.003	0.71	0.20	0.006	0.008
FA												
Intake (g/d)	508	918	385	763	499	938	44.4	<0.001	<0.001	0.61	0.01	<0.001
Crude protein												
Intake (kg/d)	3.77	3.50	2.88	2.84	2.93	2.59	0.16	<0.001	0.01	0.32	<0.001	<0.001
Duodenal flow (kg/d)	4.11	3.95	3.16	3.25	3.37	2.90	0.19	<0.001	0.08	0.10	<0.001	<0.001
Ruminal digestibility	−0.101	−0.121	−0.107	−0.148	−0.146	−0.123	0.038	0.38	0.37	0.23	0.32	0.34
Total tract digestibility	0.704	0.709	0.690	0.673	0.691	0.677	0.011	0.04	0.28	0.50	0.25	0.02

[a] Diets: EGC, early grass silage control; EGF, early grass silage fat; LGC, late grass silage control; LGF, late grass silage fat; MSC, maize silage control; MSF, maize silage fat.
[b] Feed to ileum rumen digestibility.
[c] Feed to duodenum rumen digestibility.
[d] Digestible NDF calculated as total aNDFom–INDF, digestibility calculated based on ileal DNDF flow.

Table 4
The effect of silage type and rapeseed fat supplementation on ruminal pH, VFA concentration and composition and ammonia concentration (average of 12 diurnal samples).

	Diet[a]						SEM	P_{silage}	P_{fat}	$P_{silage*fat}$	Contrast P-values	
	EGC	EGF	LGC	LGF	MSC	MSF					EG and LG *vs.* MS	EG *vs.* LG
Total VFA mmol/L	118	117	106	107	106	102	2.20	<0.001	0.37	0.35	<0.001	<0.001
VFA mol/100 mol of total VFA												
Acetic acid	61.0	60.1	59.6	60.3	54.9	55.4	0.94	<0.001	0.82	0.58	<0.001	0.48
Butyric acid	13.1	13.7	15.4	14.5	14.9	14.6	0.41	0.002	0.58	0.19	0.09	0.001
Iso-butyric acid	0.92	0.98	1.01	0.95	0.90	0.94	0.03	0.12	0.57	0.14	0.09	0.24
Propionic acid	23.1	23.2	22.2	22.6	27.1	27.0	0.73	<0.001	0.85	0.93	<0.001	0.31
Valeric acid	1.92	2.06	1.81	1.66	2.27	1.94	0.19	0.16	0.48	0.44	0.15	0.19
Acetate/Propionate ratio	2.66	2.60	2.69	2.67	2.05	2.07	0.09	<0.001	0.78	0.85	<0.001	0.50
NH_3-N (mg/100 g)	16.2	19.4	18.7	18.4	14.3	15.8	1.2	0.02	0.11	0.34	0.01	0.51
pH	6.20	6.14	6.45	6.36	6.26	6.17	0.06	<0.001	0.06	0.95	0.14	<0.001

[a] Diets: EGC, early grass silage control; EGF, early grass silage fat; LGC, late grass silage control; LGF, late grass silage fat; MSC, maize silage control; MSF, maize silage fat.

Table 5
The effect of silage type and rapeseed fat supplementation on milk production, composition and ECM and milk solids yield.

	Diet[a]						SEM	P_{silage}	P_{fat}	$P_{silage*fat}$	Contrast P-values	
	EGC	EGF	LGC	LGF	MSC	MSF					EG and LG *vs.* MS	EG *vs.* LG
Milk (kg/d)	23.4	22.8	19.9	20.3	22.7	21.5	2.39	0.21	0.73	0.90	0.73	0.09
Fat (g/kg)	42.7	45.3	46.7	48.3	48.3	45.7	2.46	0.31	0.80	0.51	0.54	0.16
Protein (g/kg)	37.5	37.4	36.2	35.9	36.3	35.8	1.31	0.01	0.39	0.87	0.09	0.01
Lactose (g/kg)	47.1	47.4	47.5	47.7	47.1	47.7	0.65	0.72	0.36	0.93	0.85	0.44
Fat (g/d)	999	1017	920	963	1144	971	125	0.48	0.64	0.49	0.33	0.49
Protein (g/d)	873	813	711	719	817	770	78.0	0.16	0.53	0.87	0.79	0.06
Lactose (g/d)	1102	1077	943	969	1065	1028	111	0.25	0.85	0.92	0.73	0.11
ECM (kg/d)	24.8	24.5	21.8	22.5	26.0	23.3	2.62	0.36	0.64	0.70	0.47	0.22

[a] Diets: EGC, early grass silage control; EGF, early grass silage fat; LGC, late grass silage control; LGF, late grass silage fat; MSC, maize silage control; MSF, maize silage fat.

Table 6
The effect of silage type and rapeseed fat supplementation on methane production per day and in relation to feed intake and digested OM, aNDFom and carbohydrate.

Item	Diet[a]						SEM	P_{silage}	P_{fat}	$P_{silage*fat}$	Contrast P-values	
	EGC	EGF	LGC	LGF	MSC	MSF					EG and LG *vs.* MS	EG *vs.* LG
CH_4												
L/day	539	474	542	505	495	455	35.9	0.06	0.01	0.72	0.03	0.37
L/kg BW	0.94	0.83	0.98	0.87	0.88	0.82	0.06	0.09	0.004	0.71	0.06	0.20
L/kg DMI[b]	29.0	27.3	31.8	31.0	26.5	25.3	1.02	<0.001	0.12	0.89	<0.001	0.004
% of GE[c] intake	6.37	5.77	6.92	6.52	5.63	5.20	0.21	<0.001	0.01	0.85	<0.001	0.005
L/kg total digested OM	43.3	38.7	51.0	48.0	40.7	40.6	1.81	<0.001	0.11	0.49	0.01	<0.001
L/kg total digested aNDFom	134	122	126	124	144	153	6.27	0.004	0.78	0.25	0.001	0.59
L/kg total digested CHO[d]	57.2	52.5	65.0	63.3	51.0	51.9	2.42	<0.001	0.37	0.52	0.002	0.002
L/kg rumen digested OM	83.0	80.8	89.3	96.0	72.7	63.7	5.25	<0.001	0.69	0.31	<0.001	0.04
L/kg rumen digested aNDFom[e]	142	131	124	129	155	141	7.91	0.05	0.31	0.46	0.03	0.23

[a] Diets: EGC, early grass silage control; EGF, early grass silage fat; LGC, late grass silage control; LGF, late grass silage fat; MSC, maize silage control; MSF, maize silage fat.
[b] DMI during CH_4 measurements.
[c] GE, gross energy.
[d] CHO, carbohydrate calculated as CHO = OM – CP – crude fat.
[e] Rumen digestibility based on ileal aNDFom flow.

4. Discussion

4.1. Grass silage maturity

With advancing maturity, the proportion of cell wall and the lignification in the plant increases, and the content of soluble carbohydrates decreases. In the present experiment, harvest was delayed three weeks for LG, and EG and LG clearly differed in NDF and lignin concentrations but still were within the range of grass silages used in practice. The grass silage in the present experiment had a slightly lower NDF concentration than those used by Rinne et al. (2002). However, delaying harvest time 3 weeks resulted in an increase in NDF concentration of around 150 g/kg DM in both experiments. The changes in chemical composition with advancing maturity reduce the plants' digestibility and feeding value and increase retention time in the rumen (Rinne et al., 1997). Consequently, DMI was reduced with advancing maturity in the present trial as also found by Rinne et al. (2002). Furthermore, ruminal and total tract aNDFom digestibilities were reduced with advancing maturity. Presumable, this was due to an increase in INDF from 30.2 in EG to 80.2 g/kg DM in LG while ruminal digestible NDF (DNDF) digestibility was not affected by maturity stage. The VFA concentration was higher for EG than for LG, probably due to more OM being fermented in the rumen on EG compared with LG.

Total daily CH_4 production did not differ between EG and LG but due to higher DMI for EG, CH_4 expressed per kg DMI or per MJ GEI was lower for EG than for LG. Boadi and Wittenberg (2002) observed that CH_4 production declined with declining forage digestibility because there was less fermentable matter available due to decreased feed intake. However, NDF intake increased with maturity in the present experiment due to higher NDF concentration in the more mature grass silage, hence resulting in a higher methanogenic potential due to induction of H_2-producing fermentative processes (Boadi et al., 2004). Furthermore, higher OM intake with less mature forage results in more fermentable matter passing the rumen due to decreased retention time and thereby reducing the proportion of feed fermented in the rumen (Huhtanen et al., 2006). Therefore, the observed increase in CH_4 production per kg DMI and per kg ECM with advancing maturity seems reasonable.

Cows on EG produced less CH_4 per kg DMI than on LG under the present conditions, where the diets were fed with the same forage to concentrate ratio. If used in practice, the LG silage could be supplemented with more concentrate than EG silage compensating for at least part of the difference in CH_4 production. However, increased concentrate feeding might not decrease the total greenhouse gas output per kg milk (Martin et al., 2010).

4.2. Starch vs. NDF digestibility

Total tract OM digestibility for grass silage and maize silage based diets was in agreement with Doreau et al. (2011), but total tract NDF digestibility was higher for our grass silage based diets. Ruminal OM digestibility was lower than observed by Owens et al. (2009) for grass and maize silage but in agreement with our earlier study (Brask et al., 2013).

Maize silage has a high starch concentration compared with grass silage where starch concentration is negligible and the major carbohydrate is NDF. The starch concentration of the used maize silage was low and below expectation as Sutton et al. (2000) and McGeough et al. (2010) reported twice as high starch concentrations at comparable DM concentration. However, there was a significant difference in starch intake and ruminal starch digestion between MS and grass silage. As duodenal starch flow was not affected by silage and rumen starch digestibility increased from 0.75 for grass silage to 0.91 for MS, it can be concluded that the starch from maize silage was highly digestible in the rumen. Starch from mature maize is reported to be less digestible in the rumen than other cereals (Doreau et al., 2011) but a high ruminal digestibility for starch from maize silage has been observed earlier (Jensen et al., 2005).

Rumen aNDFom digestibility in the present experiment was 0.70 and 0.55 for EG and MS, respectively. Juniper et al. (2008) observed lower ruminal NDF digestibility for maize silage than for grass silage. Owens et al. (2009) reported that differences between grass and maize silage NDF digestibility can be a negative effect of the greater starch intake in maize silage. Consequently, this may result in lower rumen pH, enhanced propionic acid production and less favorable conditions for fibrolytic bacteria. However, rumen pH did not differ between EG and MS. The difference in aNDFom rumen digestibility cannot be entirely attributed to the higher INDF concentration in MS, since ruminal and total tract digestibility of DNDF were still lower for MS than for EG. Lund et al. (2007) also found a lower digestibility for the potentially degradable part of NDF in maize silage compared with grass silage, in accordance with tabulated *in situ* values which show a higher concentration of potentially degradable NDF and a higher rate of degradation of grass silage compared with maize silage (NorFor, 2013).

Cows had a lower rumen pH on EG and MS than on LG, probably due to more intensive fermentation of EG compared with the more mature LG (Rinne et al., 1997) and because of the higher starch concentration in MS (Juniper et al., 2008). However, Huhtanen et al. (2006) reported that with a rumen pH above 6.2, the effect of rumen pH on fiber digestion is relatively small, therefore, the lower pH on EG and MS compared with LG has probably not affected aNDFom digestibility.

The rumen aNDFom digestibility calculated from feed intake and duodenum flow resulted in unreasonable effect of fat on MS as fat supplementation increased MS NDF digestibility, contradictory to current literature and our own earlier studies where fat either depressed NDF digestion (Martin et al., 2008) or did not affect it at all (Brask et al., 2013). Since no fiber digestion takes place in the small intestine, ruminal aNDFom digestibility was calculated from feed intake and ileum flow (Stensig et al., 1998). Feed to ileum ruminal aNDFom digestibility was in accordance with the findings of Ben Salem et al. (1993) for grass hay and maize silage rumen digestibility, indicating a numeric reduction in ruminal NDF digestibility with fat supplementation and no significant interaction between silage type and fat supplementation.

Negative small intestine digestibility was seen in four of the six diets in the present experiment but also in earlier experiments for NDF (Stensig and Robinson, 1997; Lund et al., 2007). Some studies indicate that Cr_2O_3 may be problematic as a marker (Faichney, 1972; Udén et al., 1980). However, re-calculating the results using INDF as a marker also resulted in negative values of small intestinal aNDFom digestibility.

Hind gut aNDFom digestibility was high, especially for EGF, which might indicate that rumen fermentation was limited and compensatory digestion took place in the hindgut. However, the proportion of aNDFom intake digested in the hindgut of total aNDFom digested was 0.07 for EGF which is moderate compared with up to 0.16 reported by Huhtanen et al. (2006).

4.3. The impact of forage species on CH_4 production

Total daily CH_4 production was lower for maize silage than for grass silage. The present values of CH_4 loss (between 5.2 and 6.9% CH_4 of GEI) were in agreement with earlier studies for dairy cows fed different forages (Boadi and Wittenberg, 2002). Early grass silage was more methanogenic than maize silage expressed as CH_4 per unit GEI or DMI. Some earlier studies (McCourt et al., 2007; Doreau et al., 2011) found that grass hay or silage and maize silage did not differ in CH_4 production but Staerfl et al. (2012) found a lower CH_4 production per kg DMI and per kg BW gain in fattening bulls receiving maize silage compared with grass silage.

The lower CH_4 production of MS compared with grass silage in the present experiment was presumably due to two factors: firstly, the higher starch concentration in MS diets enhanced propionate production in the rumen which is known to be an alternative hydrogen sink to CH_4 (Boadi et al., 2004). Secondly, less aNDFom was digested in the rumen for MS diets (3.29 kg *vs.* 3.91 kg for grass silages, P=0.008) despite a comparable aNDFom concentration in the diets. Acetate is a major end product of NDF fermentation (Juniper et al., 2008) and acetate production enhances CH_4 production (Johnson and Johnson, 1995). Therefore, lower rumen aNDFom digestibility contributed to lower CH_4 production for MS. Furthermore, the higher CP concentration in the grass silage than in MS could contribute to higher CH_4 production for grass silage as acetate is also produced during degradation of protein (Juniper et al., 2008). On the other hand, Kreuzer et al. (1985) found no difference in CH_4 production between cows fed 14.5 and 17.1% CP. Ramin and Huhtanen (2013) predicted a slight decrease in CH_4 production with increased CP concentration in the diet but concluded that the impact of dietary CP on CH_4 production is quantitatively of minor importance.

When CH_4 production is related to kg DMI or MJ GEI, the digestibility of nutrients is not taken into consideration, *i.e.* the intake of both DM and GE might be high but with low digestibility, nutrient excretion in the feces will be high (in the present experiment 4.75 kg OM on MS *vs.* 3.71 kg OM on EG). It has earlier been shown that reduced enteric CH_4 production can increase the methanogenic potential of the slurry (Külling et al., 2002) and a study with fecal samples from this experiment conducted by Møller (2012) showed that this was also valid here. Therefore, reducing CH_4 emission by reducing digestibility can be counteracted by increased fermentation in the slurry, which is only favorable if the slurry is used for biogas production. Methane per kg totally digested OM did not differ significantly between MS and EG, indicating that highly digestible grass silage can be as favorable as maize silage for reducing CH_4 emission. Furthermore, life cycle assessments indicate that, due to lower fertilizer use and more carbon sequestration in grassland, grass silage has a more favorable greenhouse gas balance (Doreau et al., 2011).

4.4. Fat supplementation

Supplementing the diets with cracked rapeseed resulted in a moderate FA concentration within recommendations (Beauchemin et al., 2008) and the FA composition of the rapeseed was as expected with high amounts of oleic acid. Rapeseed fat supplementation decreased CH_4 in L/day and % GEI without negative effects on intake or digestibility. This is in agreement with our and others earlier findings (Moate et al., 2011; Brask et al., 2013). The numeric reduction of CH_4 per kg DMI per percentage of fat added was on average 1.6%. This was lower than the significant 4.4% per percentage fat added in our earlier study with rapeseed fat (Brask et al., 2013) and lower than 5.6% reviewed by Beauchemin et al. (2008). However, the present result illustrates the large variation mentioned by Beauchemin et al. (2008) and is in agreement with 1.8% reduction with addition of rapeseed fat observed by Moate et al. (2011). Dong et al. (1997) found that the CH_4 reduction of rapeseed oil *in vitro* was more pronounced for a concentrate based diet than for a hay based diet. Possibly, the high forage concentration with 650 g/kg in the present trial and 630 g/kg in Moate et al. (2011) compared with 500 g/kg in Brask et al. (2013) could be an explanation for the comparatively weaker effect of fat found here.

Fat supplementation can decrease CH_4 production by lowering the quantity of OM fermented in the rumen and by influencing the microbial activity and ecosystem and, to a very minor extent, by biohydrogenation of unsaturated FA (Johnson and Johnson, 1995). In the present trial, the CH_4 production per kg digested carbohydrate did not differ and rumen fermentation was not affected by fat supplementation. Therefore, the reduction of CH_4 production was most likely due to the replacement of carbohydrates with fat that was not fermented in the rumen.

4.5. Interactions between fat supplementation and forage characteristics

There was an interaction between fat supplementation and silage type for ruminal OM digestibility, possibly because the aNDFom concentration was clearly higher in LG than in EG and therefore, the negative effect of fat on fibrolytic bacteria could

have been more important. Ben Salem et al. (1993) reported that on a fiber-rich diet, FAs would be adsorbed on particles and be more diluted in the rumen content and interact less with bacteria. However, FAs inhibit predominantly fibrolytic bacteria (Doreau and Chilliard, 1997) and consequently, OM digestion should be less sensitive to fat addition when the NDF concentration is low, which was also seen in the present trial as ruminal OM digestibility of LG was most affected by fat supplementation.

Considering the difference in starch to aNDFom ratio in the grass silages and maize silage, it is surprising that no interaction between forage and fat supplementation effect on CH_4 production was observed. Dong et al. (1997) found a difference in the effect of rapeseed fat between concentrate and hay-based diets and Chung et al. (2011) reported that linseed fat supplementation decreased CH_4 in barley silage based diets but not in grass hay. It was assumed that this interaction was observed because the additional fat affected rumen fermentation differently for starch-rich and grass-based diets (Chung et al., 2011). However, rumen fermentation was not affected by fat supplementation in the present trial, probably because of the lower fat supplementation with 27 g/kg DM additional fat compared with 100 g/kg DM by Dong et al. (1997) and because of the less harmful FA composition in rapeseed compared with linseed used by Chung et al. (2011), as monounsaturated FA are less toxic to rumen microbes than polyunsaturated FA (Giger-Reverdin et al., 2003). The CH_4 decreasing effect of fat increases with the degree of unsaturation of the FA (Giger-Reverdin et al., 2003), and rapeseed fat is rich in monounsaturated FA in contrast to linseed fat being rich in polyunsaturated FA. Furthermore, the effect of more intensive hydrogenation for fiber-rich diets is probably more important with a higher degree of unsaturation in the fat source.

5. Conclusions

An early harvest of grass can decrease enteric CH_4 production per kg DMI and per kg digested OM from dairy cows as early harvested grass has a lower NDF concentration and is more digestible than more mature plants. The use of maize silage increased the starch concentration of the diet and therewith enhanced fermentation pathways that decrease CH_4 production. Partly CH_4 is decreased by reducing the OM digestibility but the silage effect is consistent for CH_4 production per kg digested OM. Moderate fat supplementation can decrease CH_4 production without affecting digestion, and the effects of supplemented fat and forage digestibility or type on CH_4 production are additive.

Conflicts of interest

None.

Acknowledgments

The work was funded by the Danish Ministry of Food, Agriculture and Fisheries, Mælkeafgiftsfonden, and Aarhus University. The authors would like to thank Torkild Jakobsen for the skillful assistance during the experiment.

References

Åkerlind, M., Weisbjerg, M.R., Eriksson, T., Thøgersen, R., Udén, P., Ólafsson, B.L., Harstad, O.M., Volden, H., 2011. Feed analyses and digestion methods. In: Volden, H. (Ed.), NorFor – The Nordic Feed Evaluation System. Wageningen Academic Publishers, Wageningen, The Netherlands, pp. 41–54.
AOAC, 1990. Official Methods of Analysis, 15th edition. AOAC International, Gaithersburg, MC, USA.
Beauchemin, K.A., Kreuzer, M., O'Mara, F., McAllister, T.A., 2008. Nutritional management for enteric methane abatement: a review. Aust. J. Exp. Agric. 48, 21–27.
Ben Salem, H., Krzeminski, R., Ferlay, A., Doreau, M., 1993. Effect of lipid supply on in-vivo digestion in cows: comparison of hay and corn silage diets. Can. J. Anim. Sci. 73, 547–557.
Boadi, D., Benchaar, C., Chiquette, J., Masse, D., 2004. Mitigation strategies to reduce enteric methane emissions from dairy cows: update review. Can. J. Anim. Sci. 84, 319–335.
Boadi, D., Wittenberg, K.M., 2002. Methane production from dairy and beef heifers fed forages differing in nutrient density using the sulphur hexafluoride (SF6) tracer gas technique. Can. J. Anim. Sci. 82, 201–206.
Brask, M., Lund, P., Weisbjerg, M.R., Hellwing, A.L.F., Poulsen, M., Larsen, M.K., Hvelplund, T., 2013. Methane production and digestion of different physical forms of rapeseed as fat supplement in dairy cows. J. Dairy Sci. 96, 2356–2365.
Canibe, N., Højberg, O., Badsberg, J.H., Jensen, B.B., 2007. Effect of feeding fermented liquid feed and fermented grain on gastrointestinal ecology and growth performance in piglets. J. Anim. Sci. 85, 2959–2971.
Chung, Y.-H., He, M.L., McGinn, S.M., McAllister, T.A., Beauchemin, K.A., 2011. Linseed suppresses enteric methane emissions from cattle fed barley silage but not from those fed grass hay. Anim. Feed Sci. Technol. 166/167, 321–329.
Dong, Y., Bae, H.D., McAllister, T.A., Mathison, G.W., Cheng, K-J., 1997. Lipid-induced depression of methane production and digestibility in the artificial rumen system (RUSITECH). Can. J. Anim. Sci. 77, 269–278.
Doreau, M., Chilliard, Y., 1997. Digestion and metabolism of dietary fat in farm animals. Br. J. Nutr. 78, S15–S35.
Doreau, M., van der Werf, H.M.G., Micol, D., Dubroeucq, H., Agabriel, J., Rochette, Y., Martin, C., 2011. Enteric methane production and greenhouse gases balance of diets differing in concentrate in the fattening phase of a beef production system. J. Anim. Sci. 89, 2518–2528.
Faichney, G.J., 1972. Assessment of chromic oxide as an indigestible marker for digestion studies in sheep. J. Agric. Sci. 79, 493–499.
Giger-Reverdin, S., Morand-Fehr, P., Tran, G., 2003. Literature survey on the influence of dietary fat composition on methane production in dairy cattle. Livest. Prod. Sci. 82, 71–79.
Hellwing, A.L.F., Lund, P., Weisbjerg, M.R., Brask, M., Hvelplund, T., 2012. Technical note: test of a low-cost and animal-friendly system for measuring methane emissions from dairy cows. J. Dairy Sci. 95, 6077–6085.
Huhtanen, P., Ahvenjärvi, S., Weisbjerg, M.R., Nørgaard, P., 2006. Digestion and passage of fibre in ruminants. In: Sejrsen, K., Hvelplund, T., Nielsen, M.O. (Eds.), Ruminant Physiology. Wageningen, The Netherlands, Wageningen Academic Publishers, pp. 87–135.
ISO, 2008. Animal Feeding Stuffs – Determination of Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) Contents, ISO 13906:2008.

Jensen, C., Weisbjerg, M.R., Nørgaard, P., Hvelplund, T., 2005. Effect of maize silage on site of starch and NDF digestion in lactating dairy cows. Anim. Feed Sci. Technol. 118, 279–294.
Jensen, S.K., 2008. Improved Bligh & Dyer extraction procedure. Lipid Technol. 20, 280–281.
Jentsch, W., Schweigel, M., Weissbach, F., Scholze, H., Pitroff, W., 2007. Methane production in cattle calculated by the nutrient composition of the diet. Arch. Anim. Nutr. 61, 10–19.
Johnson, K.A., Johnson, D.E., 1995. Methane emissions from cattle. J. Anim. Sci. 73, 2483–2492.
Juniper, D.T., Browne, E.M., Bryant, M.J., Beever, D.E., 2008. Digestion, rumen fermentation and circulating concentrations of insulin, growth hormone and IGF-1 in steers fed diets based on different proportions of maize silage and grass silage. Animal 2, 849–858.
Knudsen, K.E.B., Åman, P., Eggum, B.O., 1987. Nutritive value of Danish-grown barley varieties I, carbohydrates and other major constituents. J. Cereal Sci. 6, 173–186.
Kreuzer, M., Müller, H.L., Kirchgessner, M., 1985. Energiebilanz und Energieverwertung bei Kühen während und nach überhöhter Proteinzufuhr. 3. Mitteilung zum Einfluss von Proteinfehlernährung bei laktierende Kühen und daraus entstehenden Nachwirkungen. Z. Tierphysiol. Tierernahr. und Futtermittelkd 54, 41–54.
Külling, D.R., Dohme, F., Menzi, H., Sutter, F., Lischer, P., Kreuzer, M., 2002. Methane emissions of differently fed dairy cows and corresponding methane and nitrogen emissions from their manure during storage. Environ. Mon. Assess. 79, 129–150.
Lund, P., Weisbjerg, M.R., Hvelplund, T., Knudsen, K.E.B., 2007. Determination of digestibility of different forages in dairy cows using indigestible NDF as marker. Acta Agric. Scand. A: Anim. Sci. 57, 16–29.
Martin, C., Morgavi, D.P., Doreau, M., 2010. Methane mitigation in ruminants: from microbe to the farm scale. Animal 4, 351–365.
Martin, C., Rouel, J., Jouany, J.P., Doreau, M., Chilliard, Y., 2008. Methane output and diet digestibility in response to feeding dairy cows crude linseed, extruded linseed, or linseed oil. J. Anim. Sci. 86, 2642–2650.
McCourt, A.R., Yan, T., Mayne, C.S., 2007. Effect of Forage Type on Methane Production from Dairy Cows. Page 48 in Proc. Br. Soc. Anim. Sci. BSAS, Penicuik, UK.
McGeough, E.J., O'Kiely, P., Foley, P.A., Hart, K.J., Boland, T.M., Kenny, D.A., 2010. Methane emissions, feed intake, and performance of finishing beef cattle offered maize silages harvested at 4 different stages of maturity. J. Anim. Sci. 88, 1479–1491.
Mertens, D.R., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J. AOAC Int. 85, 1217–1240.
Moate, P.J., Williams, S.R.O., Grainger, C., Hannah, M.C., Ponnampalam, E.N., Eckard, R.J., 2011. Influence of cold-pressed canola, brewers grains and hominy meal as dietary supplements suitable for reducing enteric methane emissions from lactating dairy cows. Anim. Feed Sci. Technol. 166/167, 254–264.
Møller, H.B., 2012. Nye foderstrategier giver mere biogas. FiB 42, 6–8 (in Danish).
NorFor, 2013. Nordic Feed Evaluation System. Feedtable, http://feedstuffs.norfor.info/ (accessed 08.01.13).
Owens, D., McGee, M., Boland, T., O'Kiely, P., 2009. Rumen fermentation, microbial protein synthesis, and nutrient flow to the omasum in cattle offered corn silage, grass silage, or whole-crop wheat. J. Anim. Sci. 87, 658–668.
Ramin, M., Huhtanen, P., 2013. Development of equations for predicting methane emissions from ruminants. J. Dairy Sci. 96, 2476–2493.
Rinne, M., Jaakkola, S., Huhtanen, P., 1997. Grass maturity effects on cattle fed silage-based diets. 1. Organic matter digestion, rumen fermentation and nitrogen utilization. Anim. Feed Sci. Technol. 67, 1–17.
Rinne, M., Huhtanen, P., Jaakkola, S., 2002. Digestive processes of dairy cows fed silages harvested at four stages of grass maturity. J. Anim. Sci. 80, 1986–1998.
Schürch, A.F., Lloyd, L.E., Crampton, E.W., 1950. The use of chromic oxide as an index for determining the digestibility of a diet. J. Nutr. 41, 629–636.
Sjaunja, L.O., Baevre, L., Junkkarinen, L., Pedersen, J., Setala, J., 1991. A Nordic Proposal for An Energy Corrected Milk (ECM) Formula. EAAP Publication 50. Performance Recording of Animals – State of the Art 1990. Centre for Agricultural Publishing and Documentation (PUDOC), Wageningen, The Netherlands, pp. 156–157.
Staerfl, S.M., Zeitz, J.O., Kreuzer, M., Soliva, C.R., 2012. Methane conversion rate of bulls fattened on grass or maize silage as compared with IPCC default values, and the long-term methane mitigation efficiency of adding acacia tannin, garlic, maca and lupine. Agric. Ecosyst. Environ. 148, 111–120.
Stensig, T., Robinson, P.H., 1997. Digestion and passage kinetics of forage fiber in dairy cows as affected by fiber-free concentrate in the diet. J. Dairy Sci. 80, 1339–1352.
Stensig, T., Weisbjerg, M.R., Hvelplund, T., 1998. Digestion and passage kinetics of fiber in dairy cows as affected by the proportion of wheat starch or sucrose in the diet. Acta Agric. Scand. A: Anim. Sci. 48, 129–140.
Stoldt, W., 1952. Vorschlag zur Vereinheitlichung der Fettbestimmung in Lebensmitteln. Fette Seifen 54, 206–207.
Sutton, J.D., Cammell, S.B., Phipps, R.H., Beever, D.E., Humphries, D.J., 2000. The effect of crop maturity on the nutritional value of maize silage for lactating dairy cows. 2. Ruminal and post-ruminal digestion. Anim. Sci. 71, 391–400.
Tilley, J.M.A., Terry, R.A., 1963. A two-stage technique for in vitro digestion of forage crops. J. Br. Grassl. Soc. 18, 104–111.
Udén, P., Colucci, P.E., Van Soest, P.J., 1980. Investigation of chromium, cerium and cobalt as markers in digesta rate of passage studies. J. Sci. Food Agric. 31, 625–632.
Weisbjerg, M.R., Hvelplund, T., 1993. Estimation of net energy content (FU) in feeds for cattle. In: National Institute of Animal Science, Report No. 3/1993, p. 39 (in Danish).