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EFFECT OF THE CARBOHYDRATE COMPOSITION OF FEED CONCENTRATATES ON METHANE EMISSION FROM DAIRY COWS AND THEIR SLURRY

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Abstract. Dietary carbohydrate effects on methane emission from cows and their slurry were measured on an individual animal basis. Twelve dairy cows were fed three of six diets each ($n = 6$ per diet) of a forage-to-concentrate ratio of 1:1 (dry matter basis), and designed to cover the cows' requirements. The forages consisted of maize and grass silage, and hay. Variations were exclusively accomplished in the concentrates which were either rich in lignified or non-lignified fiber, pectin, fructan, sugar or starch. To measure methane emission, cows were placed into open-circuit respiration chambers and slurry was stored for 14 weeks in 60-L barrels with slurry being intermittently connected to this system. The enteric and slurry organic matter digestibility and degradation was highest when offering *Jerusalem artichoke* tubers rich in fructan, while acid-detergent fiber digestibility and degradation were highest in cows and slurries with the soybean hulls diet rich in non-lignified fiber. Multiple regression analysis, based on nutrients either offered or digested, suggested that, when carbohydrate variation is done in concentrate, sugar enhances enteric methanogenesis. The methane emission from the slurry accounted for 16.0 to 21.9% of total system methane emission. Despite a high individual variation, the methane emission from the slurry showed a trend toward lower values, when the diet was characterized by lignified fiber, a diet where enteric methane release also had been lowest. The study disproved the assumption that a lower enteric methanogenesis, associated with a higher excretion of fiber, will inevitably lead to compensatory increases in methane emission during slurry storage.

Keywords: carbohydrates, dairy cows, manure storage, methane

1. Introduction

In the wake of the Kyoto Protocol in 1997, besides reducing carbon dioxide, there has also been an interest in reducing methane (CH_4) production from ruminants, and in improving its estimation, since this type of emission has been identified as the largest single source of anthropogenic CH_4, and as the second key greenhouse gas (Mathison *et al.*, 1998). Furthermore, CH_4 formation in the rumen represents an energy loss for the animal, which may vary between 2 and 12% of the animals' gross energy intake (Johnson and Ward, 1996; Giger-Reverdin and Sauvant, 2000). Based

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on the estimates used by IPCC (1996), CH_4 emission from storage of dairy cow slurry has been estimated for cool European climate to make up 12% of total CH_4 emission (110 and 14 kg head^{-1} a^{-1} for enteric and manure origin, respectively), whereas in cool climate areas in the U.S. the CH_4 emission from manure was found to be up to 23% of total CH_4 emission (118 and 36 kg head^{-1} a^{-1} for enteric and manure origin, respectively). The proportionate emission from manure storage, however, can vary widely depending on the storage type and climate, and might only represent about 1/3 of the potential yield. Since enteric CH_4 emission exceeds CH_4 emission from manure storage, the primary efforts were put on reducing enteric CH_4 emission, while manure storage has only received minor attention. However, claims to consider methane emissions in a more integrated and system-wide approach have been expressed earlier (Demeyer and Van Cleemput, 1996).

Even though enteric CH_4 emission has received more attention than CH_4 emission from manure, the existing equations for estimation are not accurate and appropriate for all feeding situations. Johnson *et al.* (1993) reported that CH_4 emissions of beef cattle fed high-concentrate diets are highly variable, comprising values between 2 and 5% of gross energy intake. According to Johnson *et al.* (1993), a lack of detailed studies is responsible for the fact that the CH_4 emission from concentrate-rich diets so far cannot be estimated as precisely by equations as that from forage-dominated diets. It was supposed by Johnson *et al.* (1993) that the existing uncertainty and the noted high variability when using concentrate-rich diets are caused by a different composition of the concentrates which, however, was not specifically analyzed and compared with other types of concentrate. Therefore, a more detailed analysis of the carbohydrate composition of the diets and of the effects of different types of carbohydrates on CH_4 formation in the animal is necessary to facilitate the accuracy of the prediction of enteric CH_4 emission from various feeding systems.

Concerning the CH_4 emission from manure storage, as far as known to the authors, no simultaneous investigations have been performed to quantify the relationship between enteric and slurry CH_4 emission on an individual cow basis. However, this could be of importance, since many attempts to suppress enteric CH_4 formation are associated with variable reductions in fiber digestibility (Moss *et al.*, 2000). This would increase the amounts of nutrients available for the microbial fermentation in the slurry, thus potentially facilitating a compensatory increase of CH_4 production as it was actually observed in a recent study (Külling *et al.*, 2002). In order to develop an overall effective mitigation strategy in animal production, focus has to be put on evaluating diets with respect to both the effect on enteric and slurry-derived methanogenesis while approaches described in literature so far mostly concentrated either on mitigation of emissions from animal or from slurry. Regression equations or dynamic mechanistic models might eventually combine both sources of CH_4 emission.

The aim of the present study was to analyze the effect of various carbohydrates in six different concentrates and to describe their effect on enteric and slurry

methanogenesis based on detailed chemical analysis, with the ultimate goal of identifying diets that reduce CH_4 emissions from the animal and the slurry. In particular, the study tested the hypothesis that a low enteric methanogenesis, possibly caused by a low-fiber degradability or a high proportion of soluble carbohydrates, is associated with a compensatorily enhanced fiber degradation and methanogenesis in the slurry.

2. Materials and Methods

2.1. Dairy cow experiment

The experiment included 12 Swiss Brown dairy cows, which were fed three out of six different experimental diets in three periods in different sequences ($n = 6$). The initial live weight of the cows was 595 ± 63 kg (mean $\pm$ S.D.) and the initial milk yield amounted to 21 ± 7 kg. The cows were allocated to the treatments before the start of the first experimental period, where first of all milk yield was considered. The cows were housed in individual tie stalls, where they had free access to water. At the very start of the experiment the cows were allowed to adapt to the tie stall barn for 8 d. The first experimental period was preceded by 5 d of gradually exchanging a standard diet consisting of a forage mixture (maize silage, grass silage and hay) and concentrate by the respective experimental diet, followed by 14 d of adaptation to the experimental diet and, finally, a period of 8 d of intensive measurement and sampling.

The six diets were formulated identical to those used in the *in vitro* experiment carried out previously (Hindrichsen *et al.*, 2004a) and consisted of forage and concentrate in a ratio of 1:1 on a dry matter (DM) basis. The forage was composed of maize silage, grass silage and hay in proportions of 0.22, 0.45 and 0.33 of DM. The six concentrates contained as specific carbohydrate sources either oat hulls, soybean hulls, apple pulp, *Jerusalem artichoke* tubers (a rare food and feedstuff), molasses or wheat, respectively (Table I). This ensured that the corresponding main carbohydrate sources in the concentrates were either lignified fiber, non-lignified fiber, pectin (measured as uronic acid polysaccharides as the backbone residue of pectin), fructans, sugars or starch. Based on tabulated values from RAP (1999), the concentrates were formulated in a way that the complete diets were similar in calculated net energy (6.2 MJ NEL kg^{-1} DM) and metabolically available protein (89 g intestinally absorbable protein kg^{-1} DM) in order to guarantee a similar milk production potential of all diets. To achieve this goal, other feedstuffs were added in varying proportions to the concentrates, and minerals completing the diet were added (Table I). The analyzed nutrient composition of the complete diets and the proportions of the individual carbohydrate fractions are also shown in Table I. The figures on the individual fibrous carbohydrates, as shown in Table I, are based on the NSP (non-starch polysaccharide) method while the mixed fractions described by neutral and acid detergent fiber were derived from the detergent analysis

TABLE I
Composition of concentrates, complete diets and the carbohydrate fraction

Experimental diets	Oat hulls diet	Soybean hulls diet	Apple pulp diet	*J. artichoke* diet	Molasses diet	Wheat diet
Ingredient composition of the concentrates (g kg^{-1} DM)						
Oat hulls	482	–	–	–	–	–
Soybean hulls	–	665	–	–	–	–
Apple pulp	–	–	514	–	–	–
Jerusalem artichoke	–	–	–	649	–	–
Sugar beet molasses	–	–	–	–	177	–
Wheat	–	–	–	–	360	437
Oat	190	16	311	–	–	–
Crystalline fat	76	47	17	–	29	55
Wheat straw meal	–	–	–	31	115	202
Grass cubes	–	172	–	141	186	153
Soybean meal	207	47	101	116	82	104
Urea	–	8	12	18	6	4
Sodium chloride	6	6	6	6	6	6
Mineral mixture[a]	39	39	39	39	39	39
Nutrient composition of the complete diets (g kg^{-1} DM)						
Organic matter	923	912	917	908	915	914
Crude protein	139	149	160	169	151	144
Ether extract	54	49	42	22	23	51
Neutral detergent fibre	513	518	401	319	355	405
Acid detergent fibre	304	365	291	205	223	238
Klason lignin[b]	134	86	132	73	87	104
Total carbohydrates[c]	596	628	583	644	654	615
Carbohydrate composition of the total carbohydrate fraction (g kg^{-1})						
Starch	161	86	259	92	277	332
Sugars	62	61	94	177	139	72
Fructan	20	22	29	186	25	24
Cellulose	339	390	286	214	225	246
Hemicelluloses	361	263	225	177	191	218
Uronic acids	40	84	63	48	37	39

[a]Providing per kg of concentrate: Ca, 6.6 g; P, 2.0 g; Mg, 1.6 g; Na, 1.6 g.
[b]Analysed with the NSP method according to Bach Knudsen (1997).
[c]Total carbohydrates = organic matter − crude protein − ether extract − Klason lignin.

(see below). The diets mostly differed in carbohydrate composition as intended, except in the soybean hull diet that presented more uronic acids than the apple pulp diet, since both soybean hulls and apple pulp are rich in pectin, but soybean hulls were included in the diet to a higher amount. The *Jerusalem artichoke* diet contained more total sugars than the molasses diet, since molasses were included in

the concentrate only to a proportion of 180 g kg^{-1}, because of practical reasons. As forage was equal for all diets, carbohydrate differences in the complete diets were only half of those found in the concentrates. Because the cows did not have exactly the same milk yield, the experimental diets had to be fed in amounts which met the individual cow's requirements for maintenance and milk production. However, the ingredient composition of the diets remained unchanged.

Each tie stall was equipped with digital electronic weighing troughs for the forage and an extra trough for the concentrate. Fresh forage and concentrate was supplied at 6.30 am, 9.30 am, 2.00 pm and 5.30 pm, except from the morning feeding where concentrates were provided 1 h after the forages.

Every day during the sampling period, urine and feces were quantitatively collected in separate containers. Urine was collected via urinals attached to the shaved skin around the vulva of the cows with the help of Velcro straps and a special glue (Cyanolit 202, 3 M AG, Rüschlikon, Switzerland). The urine flowed mainly into a 20-L container, but a sub-sample was deviated into a 1-L container containing 20 mL of 5 M sulfuric acid to keep the pH under 5 for the nitrogen conservation. All urine sub-samples were frozen at −20 °C. The total fecal output was collected in steel trays, which were placed underneath the cow. The total amounts of feces were weighed daily and sub-samples were taken and frozen immediately at −20 °C. At the end of the sampling period, the daily samples of feces were mixed in aliquot amounts. A sub-sample of that was frozen again, while the residual amount was dried at 60 °C for 72 h and milled through a 0.75-mm sieve. Similarly, non-acidified and acidified urine were mixed in aliquot amounts at the end of the sampling period and the samples were frozen until analysis. Feed samples were taken once a week while sub-samples of feed residues were taken every day. Both feed samples and residues were dried at 60 °C for 48 h and milled through a 0.5-mm sieve.

On the fourth and fifth day of each sampling period the gaseous exchange of the cows was measured in two open-circuit respiration chambers (Sutter and Beever, 2000). The chambers were also equipped to completely collect feces and urine, and allowed milking of the cows similar as in the tie stall. Each chamber had a volume of 20 m^3 and was air-conditioned with a constant humidity (58.7 ± 2.3%), temperature (18.1±1.0 °C), air flow (37.4±0.4 m^3 hr^{-1}) and pressure (996±8 hPa). Before starting the measurements the gas analyzers were manually calibrated with reference gases. The concentration of CH_4, carbon dioxide and oxygen in the air flowing into the chambers was measured every 20 min for 100 s, while the gas composition of the air flowing out of the two chambers was measured alternatively for 100 s each over the following 20 min. Then the cycle started again. Every fourth cycle an automatic calibration with reference gases was done. Concentrations of CH_4 and carbon dioxide were measured with an infrared analyzer (NGA 2000, Fisher-Rosemount, Ohio, USA), while the concentration of oxygen was determined with an Oxymat 6 (Siemens AG, Karlsruhe, Germany). The air volume leaving the chambers was continuously recorded with in-line electronic flow meters (Swingwirl

DV 630; Flowtec AG, Reinach, Switzerland). The gaseous exchange measurements were conducted for 22.5 h d^{-1}.

To meet the Swiss guidelines for animal welfare (Bundeskanzlei, 2003), the cows were allowed to walk in an outdoor yard with concrete floor every other day, except during the sampling period.

2.2. Slurry storage experiment

For the slurry storage experiment, 2 kg of feces and 1 kg of urine were collected daily during the 8 d of sampling period from each cow and frozen at −20 °C. About 10 kg of feces and urine were combined in the proportions excreted and mixed with 5 kg of water, a mixture from now on referred to as slurry. The water was added to simulate typical situations on Swiss farms (Külling *et al.*, 2001). A new technique, differing from that described by Külling *et al.* (2001) and applied in Külling *et al.* (2002), was developed and intensively tested before use. This confirmed comparability of the two methods and the possibility to use previously frozen slurry samples. Accordingly, storage now took place in 60-L barrels, which were covered with air-tight lids. In the center of each lid a hose (0.5 cm in diameter) connected the barrel with the open-circuit respiration chamber equipment in an air-tight way. On the side, 35 cm above ground of the barrel, another hose was attached, with the other end being placed outside of the building to suck in air from the outside (0.34 ± 0.07 m^3 h^{-1}). For 14 weeks, CH_4 emission from each barrel was measured once a week over 9 h. This particular measurement period was chosen since CH_4 emission from slurry may not be completed earlier (Külling *et al.*, 2002). The room was kept sealed as good as possible, which allowed maintaining a relatively constant temperature (23.6 ± 1.3 °C). At the end of storage, residues were homogenized and two samples were taken. One sub-sample was used for fiber analyses and was dried at 60 °C for 72 h and ground through a 0.75-mm sieve. The slurry sample taken at the beginning of storage and the second sub-sample collected at the end of storage were immediately analyzed for contents of nitrogen (N), ammonium-N, total DM and total organic matter (OM).

2.3. Chemical analyses

Total DM (105 °C) and ash (550 °C) analyses were performed on an automatic analyzer (TGA-500, Leco Corporation, St. Joseph, Michigan, USA). Silica sand was added to the crucible when measuring DM contents of urine, feces and slurry. Because the results on slurry DM correlated closely with those calculated from the separate analysis of feces and urine ($r = 0.926$), slurry DM data were used for all further calculations. Nitrogen analysis (C/N analyzer, Leco-Analysator Typ FP-2000, Leco Instrumente GmbH, Kirchheim; crude protein = 6.25 × N) was done in feed, fresh feces and acidified urine. The ammonium-N concentration of slurry

samples was determined as total ammonium N by MgO distillation (Distillation Unit 323, Büchi, Flawil, Switzerland) as described by Amberger *et al.* (1982). The mass balance method was applied to calculate emissions of total N from slurry and the changes in ammonia N. Ether extract (Soxhlet method; Büchi Universal Extraction System B-811, Flawil, Switzerland) was only analyzed in feed.

Feed, dried feces and slurries were analyzed for neutral detergent fiber (NDF) and acid detergent fiber (ADF) on a Fibertec (Fibertec System M, Tecator, 1020 Hot Extraction, Flawil, Switzerland). For NDF analysis, α-amylase was included as suggested by Van Soest *et al.* (1991), while ADF was analyzed as described in AOAC (1990, No. 973.18 C).

A more detailed analysis of carbohydrates was performed in forages and concentrates. Soluble and insoluble non-starch-polysaccharides (NSP) were determined by measuring the constituent monomeric sugars (rhamnose, fucose, arabinose, xylose, mannose, galactose, glucose) as alditol acetates with gas-liquid chromatography and the uronic acids colorimetrically using a combination of the Englyst *et al.* (1982) and the Uppsala method (Theander *et al.*, 1994) as described by Bach Knudsen (1997). Klason lignin was measured gravimetrically as the insoluble residue after 12 M sulfuric acid treatment (Theander *et al.*, 1994). On the basis of the results of the NSP method, cellulose and hemicelluloses were calculated (Bach Knudsen, 1997) according to the following formulae:

(1) $\text{Cellulose} = \text{NSP}_{\text{glucose}\,(12\,\text{mol}\,\text{L}^{-1})} - \text{NSP}_{\text{glucose}\,(2\,\text{mol}\,\text{L}^{-1})}$
(2) $\text{Total NCP} = \text{rhamnose} + \text{fucose} + \text{arabinose} + \text{xylose} + \text{mannose} + \text{galactose} + \text{glucose} + \text{uronic acids}$
(3) $\text{Hemicelluloses} = \text{total NCP} - \text{total uronic acids}.$

Starch contents were analyzed by an enzymatic colorimetric method (Bach Knudsen *et al.*, 1987). Low-molecular-weight sugars (sucrose, glucose, fructose) and fructan were measured by the enzymatic colorimetric method described by Larsson and Bengtsson (1983). In *Jerusalem artichoke* the procedure described in Hindrichsen *et al.* (2004a) was applied in order to avoid over-estimation of sucrose and under-estimation of fructan given by the analysis of low-molecular weight sugars and fructan.

2.4. Statistical evaluation

In the data evaluation by analysis of variance, dietary treatment and experimental period were considered as effects. The data were subjected to the general linear model (GLM) procedure of the SAS program (version 8.2, SAS Institute Inc., Cary, NC, USA). One dataset of one cow having large refusals of the apple pulp concentrate was excluded from the evaluation. Multiple comparisons among means were carried out by the Tukey method. In the tables the respective treatment means ($n = 6$) are given. Multiple regression analysis was carried out with the MAXR

(maximum *R*-squared improvement) step-wise procedure of SAS to determine the relationship between nutrient supply and methanogenesis.

3. Results

3.1. Feed intake, excretion and effect of slurry storage

Total DM and OM intake with feed did not differ significantly among diets (Table II). The same was true for milk yield which accounted for 18.0, 20.8, 15.9, 18.5, 19.0 and 19.2 kg in the diets characterized by oat hulls, soybean hulls, apple pulp, *Jerusalem artichoke*, molasses and wheat, respectively. Intake of DM and milk yield had a correlation of 0.95. There were significant differences for NDF and ADF intakes which were high with the soybean hulls and oat hulls diet compared to the other diets. The amount of excreta and of nutrients lost with the excreta varied

TABLE II

Total intake of nutrients, average daily excretions of dairy cows and slurry residues after 14 weeks of storage (kg cow^{-1} d^{-1}, $n = 6$)

Experimental diets	Oat hulls diet	Soybean hulls diet	Apple pulp diet	*J. artichoke* diet	Molasses diet	Wheat diet	S.E.M.	*P*-value
Original matter								
Intake with feed	23.41	25.69	20.94	24.03	23.18	24.36	1.949	0.661
Excretion with urine	14.04 *b*	18.18 *ab*	13.82 *b*	20.12 *a*	18.49 *ab*	14.50 *b*	1.188	0.002
Excretion with feces	32.48	40.72	28.92	37.91	34.48	35.81	3.560	0.336
DM								
Intake with feed	16.07	17.29	14.19	16.23	15.48	16.40	1.344	0.702
Total excretion	7.66 *a*	6.81 *ab*	5.22 *b*	5.43 *ab*	5.50 *ab*	5.94 *ab*	0.547	0.026
Slurry storage residue	5.62 *a*	4.67 *ab*	3.55 *b*	3.68 *b*	3.84 *b*	4.12 *b*	0.345	0.002
OM								
Intake with feed	14.82	15.76	13.00	14.75	14.18	14.99	1.237	0.729
Total excretion	6.61 *a*	5.57 *ab*	4.22 *b*	4.27 *b*	4.42 *b*	4.83 *ab*	0.465	0.007
Slurry storage residue	4.63 *a*	3.48 a*b*	2.63 *b*	2.54 *b*	2.73 *b*	3.04 *b*	0.278	<0.001
NDF								
Intake with feed	8.23 *a*	8.97 *a*	5.69 *b*	5.15 *b*	5.46 *b*	6.63 *ab*	0.589	<0.001
Total excretion	4.80 *a*	3.55 *ab*	2.75 *b*	2.73 *b*	2.82 *b*	2.95 *b*	0.319	<0.001
Slurry storage residue	3.29 *a*	1.91 *b*	1.67 *b*	1.44 *b*	1.61 *b*	1.70 *b*	0.189	<0.001
ADF								
Intake with feed	4.87 *ab*	6.31 *a*	4.12 *b*	3.31 *b*	3.43 *b*	3.88 *b*	0.362	<0.001
Total excretion	2.82 *a*	2.57 *ab*	2.11 *ab*	1.71 *b*	1.74 *b*	1.84 *b*	0.204	0.002
Slurry storage residue	2.09 *a*	1.46 *b*	1.41 *b*	1.12 *b*	1.21 *b*	1.29 *b*	0.127	<0.001
Klason lignin								
Intake with feed	2.14 *a*	1.48 *bc*	1.86 *ab*	1.18 *c*	1.35 *bc*	1.70 *abc*	0.135	<0.001
Total excretion	1.71 *a*	1.36 *ab*	1.36 *ab*	0.97 *b*	1.20 *b*	1.29 *ab*	0.112	0.005

Different letters in each row represent significant ($P < 0.05$) difference.

significantly among the experimental diets. The oat hulls diet caused the highest DM, OM, NDF and ADF excretion relative to all other diets. After 14 weeks of storage, the slurries from cows fed the oat hulls diet still contained the significantly highest ($P < 0.05$) amounts of DM, OM, NDF and ADF while differences among the other diets were relatively smaller. Slurry weights were reduced to two thirds of initial during 14 weeks of storage (data not shown), and a crust had formed on all slurries.

In Table III the apparent digestibility and degradability of DM, OM, NDF and ADF in animal and during 14 weeks of slurry storage is shown. The proportions of nutrients apparently digested were significantly ($P < 0.01$) different among experimental diets. Cows fed the *Jerusalem artichoke* diet showed the highest apparent DM and OM digestibility while these were lowest in cows fed the oat hulls diet. The apparent NDF and ADF digestibility were significantly ($P < 0.05$) higher in cows fed the soybean hulls diet compared to the other diets, with differences of as high as 40 and 37% compared to the oat hulls diet, respectively. The dietary differences in proportionate nutrient degradation from the stored slurry were similar to those found in the cows. Degradation of DM and OM, but also of NDF, was highest in the slurry of cows fed the *Jerusalem artichoke* diet, while ADF degradation was highest with the soybean hulls diet. The slurry of cows fed the oat hulls diet expressed the proportionately lowest nutrient degradation. Total DM and NDF

TABLE III

Extents of apparent nutrient digestibilities in the animal and degradation in the slurry during 14 weeks of storage in relation to the respective amounts initially available ($n = 6$)

Experimental diets	Oat hulls diet	Soybean hulls diet	Apple pulp diet	*J. artichoke* diet	Molasses diet	Wheat diet	S.E.M.	*P*-value
Apparent nutrient digestibility in the animal								
DM	0.566 *d*	0.656 *c*	0.677 *bc*	0.722 *a*	0.694 *ab*	0.679 *bc*	0.0078	<0.001
OM	0.586 *d*	0.684 *c*	0.701 *bc*	0.744 *a*	0.720 *ab*	0.704 *bc*	0.0077	<0.001
NDF	0.468 *c*	0.656 *a*	0.576 *b*	0.563 *b*	0.556 *b*	0.604 *ab*	0.0138	<0.001
ADF	0.472 *c*	0.646 *a*	0.550 *b*	0.568 *b*	0.563 *b*	0.564 *b*	0.0146	<0.001
Nutrient degradation in the slurry								
DM	0.244 b	0.361 *a*	0.317 *ab*	0.391 *a*	0.350 *a*	0.313 *ab*	0.0227	0.003
OM	0.294 c	0.436 *ab*	0.398 *b*	0.501 *a*	0.450 *ab*	0.398 *b*	0.0202	<0.001
NDF	0.296 c	0.503 *a*	0.393 *b*	0.526 *a*	0.473 *ab*	0.431 *ab*	0.0213	<0.001
ADF	0.235 c	0.477 *a*	0.335 *abc*	0.416 *ab*	0.356 *abc*	0.286 *bc*	0.0362	<0.001
Total nutrient degradation (animal & slurry storage)								
DM	0.639 *c*	0.749 *b*	0.749 *b*	0.799 *a*	0.770 *ab*	0.749 *b*	0.0103	<0.001
OM	0.742	0.832	0.815	0.845	0.795	0.817	0.0310	0.277
NDF	0.625 *c*	0.829 *a*	0.743 *b*	0.794 *ab*	0.765 *b*	0.774 *b*	0.0128	<0.001
ADF	0.636 *ab*	0.843 *a*	0.715 *ab*	0.686 *ab*	0.608 *b*	0.662 *ab*	0.0517	0.048

Means with unequal letters in each row are significantly different ($P < 0.05$).

degradation in animal and from slurry was significantly ($P < 0.05$) lower for cows fed the oat hulls diet compared to the five other diets. The total NDF and ADF degradation were highest for cows fed the soybean hulls diet. The extent of ADF degradation was lowest in cows fed the molasses diet, but this was only significant against the soybean hulls diet.

3.2. Effect of diet on methane emission from the cows and their slurry

There was no significant ($P > 0.05$) difference across diets in enteric CH_4 emission per cow per day (Table IV), per kg of daily milk production and per kg of metabolic live weight ($W^{0.75}$; data not shown). The cows differed in milk yield and, consequently, DM intake varied as the amount offered had been adapted without changing the diet composition in order to meet their individual requirements. The DM intake was closely correlated with the enteric CH_4 emission [enteric CH_4 (g) = 17.1 × DM intake (kg) + 97.4; $r = 0.842$; $P < 0.001$, $n = 35$]. Methane emission per kg of DM intake from the oat hulls diet was lower ($P < 0.05$) compared to the other diets, except from the *Jerusalem artichoke* diet. There were also differences in CH_4 emission related to the amount of NDF digested, decreasing in the order of *Jerusalem artichoke*, molasses, apple pulp, wheat, oat hulls and soybean hulls diet.

Including daily intakes of cellulose, hemicelluloses, lignin (ADL), starch and sugars to regression analysis, the following equation for enteric CH_4 emission was calculated with the MAXR procedure of SAS ($R^2 = 0.794$; $n = 35$):

$$\text{Enteric } CH_4 \text{ (g d}^{-1}) = 84 + 47 \times \text{cellulose (kg d}^{-1}) + 32 \times \text{starch (kg d}^{-1}) + 62 \times \text{sugars (kg d}^{-1}).$$

Intakes of hemicelluloses and ADL did not significantly ($P > 0.05$) contribute to and did not clearly improve the equation which is why they were excluded. The same procedure used for total CH_4 emission from enteric fermentation and slurry storage resulted in the following equation ($R^2 = 0.879$, $n = 35$), where also lignin and hemicelluloses were significant ($P < 0.05$):

$$\text{Total } CH_4 \text{ (g d}^{-1}) = 123 + 84 \times \text{cellulose (kg d}^{-1}) - 30 \times \text{hemicelluloses (kg d}^{-1}) + 58 \times \text{starch (kg d}^{-1}) + 73 \times \text{sugars (kg d}^{-1}) - 95 \times \text{lignin (kg d}^{-1}).$$

In a second approach, amounts of actually digested nutrients such as cellulose, hemicelluloses, starch and sugar were included in the MAXR procedure of SAS. The ADL was not included, since it is expected to be indigestible and extremely slowly fermentable. Starch and sugars were assumed to be completely digested by

TABLE IV
Methane emission from enteric fermentation and slurry storage over 7 or 14 weeks ($n = 6$)

Experimental diets	Oat hulls diet	Soybean hulls diet	Apple pulp diet	*J. artichoke* diet	Molasses diet	Wheat diet	S.E.M.	*P*-value
Enteric methane emission								
g cow^{-1} d^{-1}	330	429	351	377	382	409	25.8	0.109
g kg^{-1} DM intake	20.7 *b*	25.0 *a*	24.8 *a*	23.6 *ab*	24.8 *a*	25.3 *a*	0.85	0.006
g kg^{-1} digested OM	54	56	54	49	52	55	1.9	0.195
g kg^{-1} digested NDF	87 *de*	74 *e*	107 *bc*	133 *a*	127 *ab*	104 *cd*	4.6	<0.001
g kg^{-1} milk protein	549	615	614	604	552	645	51.1	0.729
Methane emission from slurry								
7 weeks of storage, g cow^{-1} d^{-1}	17.6	25.8	28.1	34.5	44.7	39.4	8.51	0.281
14 weeks of storage, g cow^{-1} d^{-1}	63.7	113.0	73.7	90.7	105.4	103.4	17.94	0.072
7 weeks of storage, % of total CH_4	5.2	5.9	7.4	7.5	10.8	8.4	1.76	0.305
14 weeks of storage, % of total CH_4	16.0	20.8	17.4	19.1	21.9	19.8	2.15	0.422
14 weeks of storage g kg^{-1} DM intake	4.0	6.7	5.2	5.6	6.9	6.3	0.71	0.076
14 weeks of storage, g kg^{-1} DM in week 0	8.5 *b*	15.9 *a*	14.1 *ab*	15.0 *ab*	18.1 *a*	16.9 *a*	2.03	0.007
14 weeks of storage g kg^{-1} degraded OM	33	43	42	36	49	52	5.0	0.117
14 weeks of storage g kg^{-1} degraded NDF	47 *b*	61 *ab*	68 *ab*	58 *ab*	74 *ab*	79 *a*	6.8	0.035
Total (enteric and slurry) methane emission								
7 weeks of storage, g cow^{-1} d^{-1}	348	454	379	449	426	412	28.40	0.089
14 weeks of storage, g cow^{-1} d^{-1}	394 *b*	542 *a*	424 a*b*	468 a*b*	487 a*b*	513 a*b*	31.30	0.023
g kg^{-1} DM intake	25 *b*	32 *a*	30 *a*	29 *a*	32 *a*	32 *a*	0.9	<0.001
g kg^{-1} total degraded OM	37	42	40	38	44	43	2.46	0.310
g kg^{-1} total degraded NDF	78 *c*	74 *c*	101 *b*	116 *ab*	117 *a*	102 a*b*	3.72	<0.001
g kg^{-1} milk protein	628	763	726	731	758	751	72.24	0.783

Means with unequal letters in each row are significantly different ($P < 0.05$).

the animal and therefore had not been measured in feces. For enteric CH_4 emission alone, hemicelluloses were taken into the equation as the first variable followed by sugars. The MAXR procedure made two suggestions for a three-variable equation, where the equation with sugars, starch and cellulose had a slightly higher coefficient of regression than the equation including hemicelluloses, cellulose and sugars. The highest R^2 (0.843; $n = 35$) was achieved with four variables including cellulose,

hemicelluloses, starch and sugars:

$$\begin{aligned}\text{Enteric } CH_4 \text{ (g d}^{-1}) = {} & 91 + 50 \times \text{digested cellulose (kg d}^{-1}) \\ & + 40 \times \text{digested hemicelluloses (kg d}^{-1}) \\ & + 24 \times \text{digested starch (kg d}^{-1}) \\ & + 67 \times \text{digested sugars (kg d}^{-1}).\end{aligned}$$

The corresponding equation for total CH_4 emission ($R^2 = 0.832$; $n = 35$) was:

$$\begin{aligned}\text{Total } CH_4 \text{ (g d}^{-1}) = {} & 108 + 46 \times \text{degraded NDF (kg d}^{-1}) \\ & + 30 \times \text{degraded starch (kg d}^{-1}) \\ & + 85 \times \text{degraded sugars (kg d}^{-1}).\end{aligned}$$

In this equation, the fiber fraction could not be distinguished into hemicelluloses and cellulose, since ADL was not measured in the slurry after 14 weeks of storage.

With all dietary treatments, the CH_4 emission from the slurries increased shortly after the start of storage and reached its peak between weeks 6 and 9 (Figure 1). During the peak period, the slurry of cows receiving the soybean hulls diet had a level of CH_4 emission twice as high as that of cows fed the oat hulls diet, but the difference was not significant. Only the CH_4 emission from cows getting the molasses diet was significantly ($P < 0.05$) higher in weeks 5 and 6 compared to that of cows receiving the oat hulls diet. The total quantity of CH_4 released from the

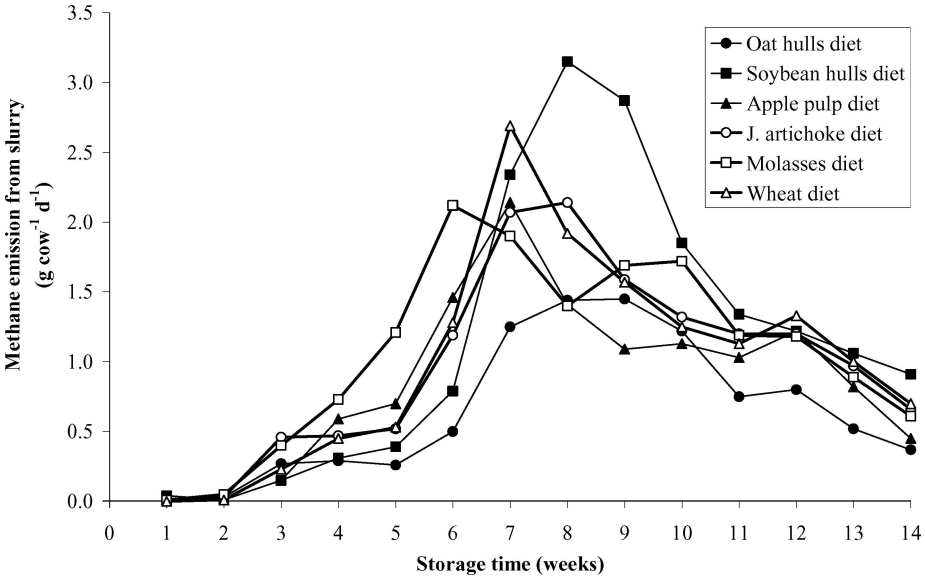

Figure 1. Methane emission from slurry over a storage period of 14 weeks as influenced by different experimental diets fed to dairy cows ($n = 6$ per dietary treatment).

slurry did not show significant treatment effects after 7 weeks, but after 14 weeks treatment effects were approaching significance ($P = 0.072$).

When relating CH_4 emission during 14 weeks of storage to kilograms of initial slurry DM, a clear difference ($P < 0.01$) was observed among the experimental groups (Table IV). The slurry originating from the molasses diet expressed the highest CH_4 emission, followed by wheat, soybean hulls, *Jerusalem artichoke*, apple pulp and oat hulls diets. The proportion of CH_4 produced in the slurry compared to the amount totally emitted (enteric & slurry) ranged between 5.2 and 10.8% in the first 7 weeks and between 16.0 and 21.9% after 14 weeks of storage, and there were no significant treatment effects. There was a positive, although not significant, correlation ($r = 0.08$) between enteric and slurry (14 weeks) CH_4 emission per kg of DM intake. The lack of a closer relationship was mainly due to high variation in CH_4 emission from the slurry compared to the lower variation in enteric CH_4 emission. Overall, the data for total CH_4 emission showed similar treatment differences as the data of enteric CH_4 emission alone.

Similar to N intake, the total daily N excretion of the cows did not significantly differ among experimental groups (Table V), while the percentage of total nitrogen excreted via urine was significantly higher for cows fed the oat hulls diet compared to cows fed the molasses diet ($P < 0.05$). Slurry DM contents before and after storage differed among diets reflecting both different fecal DM contents and varying urine volumes. The slurry clearly differing from all others was the one obtained from the oat hulls diet, which was characterized by a high DM content before and after storage for 14 weeks. The initial slurry N content was significantly ($P < 0.05$) lower for cows fed the oat hulls diet compared to the other five diets. After 14 weeks of storage the N content of the slurry of this treatment was only significantly ($P < 0.05$) lower compared to cows fed the *Jerusalem artichoke* diet. Initially, the ammonium-N content of the slurry from oat hulls-fed cows was higher ($P < 0.05$) compared to that of the other diets, except from the *Jerusalem artichoke* treatment. Storing the slurries for 14 weeks resulted in an adjustment of ammonium-N content to lower levels ranging between 209 and 311 g kg^{-1} total N independent of the dietary treatment. The total N loss during the 14 weeks of storage related to the initial slurry N content was not significantly influenced by the different diets similar to the gaseous N loss from the daily amount of slurry produced by individual cows.

4. Discussion

Different from many previous animal studies also investigating enteric carbohydrate digestion and methanogenesis, various carbohydrate sources either common or rare, though mostly available in public trade, were fed to cows by changing only the composition of the concentrates at a constant forage composition and proportion. This allowed the evaluation of the carbohydrate effects at a presumed unchanged

TABLE V

Nitrogen intake and excretion and slurry N and ammonium-N content as well as N loss during 14 weeks of storage ($n = 6$)

Experimental diets	Oat hulls diet	Soybean hulls diet	Apple pulp diet	*J. artichoke* diet	Molasses diet	Wheat diet	S.E.M.	*P*-value
Nitrogen intake and excretion								
N intake (g cow^{-1} d^{-1})	357.4	410.0	361.6	438.1	375.9	378.8	2.45	0.520
Urine N (g cow^{-1} d^{-1})	98.7	85.5	72.5	102.5	71.6	76.4	7.60	0.029
Feces N (g cow^{-1} d^{-1})	119.4	164.1	128.9	133.5	142.7	120.6	14.90	0.308
Milk N (g cow^{-1} d^{-1})	95.2	109.6	92.7	111.8	109.2	108.9	13.14	0.851
Total excreta N (% of N intake)	61.3	61.1	55.9	54.1	56.5	53.8	3.38	0.468
Urine N (% of total N excretion)	45.4 *a*	34.8 *ab*	36.4 *ab*	44.1 *ab*	34.1 *b*	39.3 *ab*	2.58	0.016
Slurry composition								
DM at week 0 (g kg^{-1})	109.7 *a*	84.2 *b*	83.4 *bc*	75.9 *bc*	75.3 *c*	82.6 *bc*	1.92	<0.001
DM at week 14 (g kg^{-1})	279.7 *a*	132.0 *b*	147.9 *b*	101.7 *b*	123.5 b	124.8 *b*	1.67	<0.001
Total N at week 0 (g kg^{-1} DM)	29.7 *b*	39.3 *a*	44.2 *a*	45.7 *a*	40.2 *a*	38.9 *a*	1.56	<0.001
Total N at week 14 (g kg^{-1} DM)	16.1 *b*	29.0 *ab*	28.2 *ab*	40.6 *a*	26.3 *b*	26.3 *b*	3.12	<0.001
NH_4^+-N at week 0 (g kg^{-1} N)	542 *a*	333 *b*	388 *b*	444 *ab*	332 *b*	381 *b*	27.5	<0.001
NH_4^+-N at week 14 (g kg^{-1} N)	223	259	209	311	262	268	25.7	0.161
Total N loss during 14 weeks of storage								
N loss, g kg^{-1} initial slurry N	591	525	565	471	573	538	40.5	0.429
N loss, g cow^{-1} d^{-1} from slurry	131	148	130	132	134	125	14.9	0.922

Means with unequal letters in each row are significantly different ($P < 0.05$).

ruminal pH, a key to avoid disturbing the sensitive fiber degrading microbes. However, this approach, together with the goal of producing isoenergetic diets which could be fed to cows and meet their individual demand meant that the differences among the six experimental diets were comparably small and not provoking the maximum response in nutrient degradation and methanogenesis possible. There were relatively large variations among groups in milk yield and, associated with that, in DM intake which limits comparability of absolute group differences in methanogenesis. Relating CH_4 data to DM intake and to digested OM and NDF widely excluded this bias.

4.1. Nutrient degradation in animal and slurry

The nutrient composition of the experimental slurries differed between the treatments for two reasons: (i) the diets affected the nutrient content and composition

of the excreta, and (ii) feces and urine were mixed in the proportions reflecting the excretory patterns of the individual animals while most other studies used a standard feces-to-urine ratio. With regard to the different experimental diets fed, the cows receiving the oat hulls diet, characterized by highly lignified fiber, showed the lowest extents of apparent digestibility of OM, NDF and ADF in the digestive tract, as also observed in other studies (Jung *et al.*, 1997). This trend continued during slurry storage for 14 weeks. This was presumably due to the high degree of lignification of the fiber in the oat hulls, which made it widely inaccessible for degradation by the microorganisms present in the digestive tract of the animal or in the slurry. The apple pulp diet, characterized by pectin and presenting a similar lignin content as the oat hulls diet, also resulted in a low-fiber degradation in the slurry but not in the animal. This suggests that lignin in the apple pulp diet, other than in oat hulls diet, is less homogenously distributed and thus associated with a smaller part of the fiber fraction. This would allow the animal's microbes to digest the non-lignified part of the fiber, leaving the slurry microbes with the lignified part. The extent of apparent digestibility and degradation of fiber from the diet characterized by easily-degradable fiber from soybean hulls was high both in the animal and during slurry storage. The use of the inulin-enriched diet, by contrast, caused a low fiber degradation in the animal compared to that found with the soybean hulls diet. This may have been the result of the high sugar content of the *Jerusalem artichoke*, which has been shown to impair fiber degradation (Khalili and Huhtanen, 1991), although this was probably less pronounced when varying carbohydrates exclusively in the concentrate (see below). The fiber not degraded in the rumen when feeding the *Jerusalem artichoke* diet was subsequently degraded in the slurry.

4.2. Enteric methane emission

The findings on the CH_4 formation per unit of digested NDF in the present study correlated strongly ($r = 0.99$) with the results of a preliminary *in vitro* study examining similar diets *in vitro* with the Rumen Simulation Technique (Hindrichsen *et al.*, 2004a). Digestible fiber is commonly considered to be the major contributor to methanogenesis (Moss *et al.*, 2000). The findings of the present experiment supported this only when CH_4 release per unit of digested fiber was calculated. The regression analysis demonstrated that in this case sugar intake has a larger impact on the daily CH_4 production than cellulose and hemicelluloses. Also at the level of actually digested nutrients, the dietary sugars still had the highest impact on the daily CH_4 production, higher than digested fibrous carbohydrates. Even though the sugar rich diets reduced the fiber digestibility, there were still enough fermentation end products available for the methanogenic Archaea to produce a fairly high amount of CH_4. This could be explained by the observed stimulation of a high molar proportion of butyrate by soluble sugars compared to starch (Moloney *et al.*, 1994; Stensig *et al.*, 1998; Hindrichsen *et al.*, 2004a). Butyrate, together with acetate, is

assumed to promote methanogenesis in the rumen, while propionate formation is a competitive pathway for hydrogen utilization and will be stimulated when feeding predominantly starch (Moss *et al.*, 2000). However, the high methanogenic effect of sugar might be limited to situations where a high ruminal pH still guarantees an undisturbed Archaeal activity (Russell, 1998; extensively discussed in Hindrichsen *et al.*, 2004a) while, at higher intake, structural carbohydrates could be more methanogenic than sugar (Torrent *et al.*, 1994).

None of the equations used for CH_4 estimation as quoted by Wilkerson *et al.* (1995) differentiated for starch and sugars when considering the influence of the soluble carbohydrates on enteric CH_4 emission. However, also other authors have been aware of a potentially important impact of dietary sugars on the ruminants' CH_4 emission. Johnson and Ward (1996) gave an equation (recalculated from Mcal d^{-1}) from unpublished data based on the amounts of nutrients digested in the rumen, saying: CH_4 (g d^{-1}) = 41 + 30× digested sugars (kg d^{-1}) + 6× digested starch (kg d^{-1}) + 51× digested cell walls (kg d^{-1}). Based on the values of Bannink *et al.* (2000), Monteny and Bannink (2004) calculated that sugars, starch, hemicellulose, cellulose and protein in the rumen of lactating cows on average yield 3.40, 2.35, 2.98, 3.40, and 1.24 kJ CH_4 per g of substrate fermented, respectively. Furthermore, in their mechanistic model, Mills *et al.* (2001) simulated a range of concentrates varying in starch-to-sugar ratio and predicted that the CH_4 production will decrease by 14.7% when starch instead of sugars is used. The particular effect of sugar on the CH_4 emission was first mentioned by Beever (1993) when quoting different theoretical stoichiometric equations for diets characterized by sugar, starch and forage. However, no *in vivo* experiments had been set up to confirm these calculations. The difference in methanogenic potential between starch and sugars could be even more pronounced in cases where more resistant forms of starch such as maize or potato starch are fed instead of wheat starch, which was used here. The different coefficients found by Johnson and Ward (1996) as well as Bannink *et al.* (2000) on one hand and the present study on the other hand might be due to the data they are based on. A comparison of the estimated CH_4 emission by the mechanistic model of Mills *et al.* (2001) and the average findings of the present study resulted in a fairly close correlation ($r = 0.664$) even though the average value of the starch-rich diet was an outlier (Hindrichsen *et al.*, 2004b).

In the present regression analysis, the impact of dietary cellulose and hemicelluloses on daily enteric CH_4 formation was much greater when related to apparently degraded amounts since the negative influence of lignin was excluded. Lignin prevents fiber degradation and therefore reduces methanogenesis per unit of fiber consumed. Although not significant for enteric methanogenesis alone, increasing lignin amounts significantly reduced total system methanogenesis in the present study. The importance of lignin for the estimation of ruminant CH_4 emission was also shown in the data compiled by Giger-Reverdin and Sauvant (2000). As a consequence for the development of equations for CH_4 estimation, either equations have to be based on digestible nutrients or dietary lignin has to be considered. Apart from

that, the present equations based on variations in concentrate composition have to be refined by data obtained with different forage proportions and types.

4.3. METHANE EMISSION FROM THE SLURRY AND TOTAL SYSTEM METHANE EMISSION

This experiment focused on the effect of different diets on the enteric methane emission and the corresponding methane emission from slurry storage as a very common form of storage. This should be widely equivalent to total losses of this system as under the aerobic conditions during slurry application a significant methane formation is unlikely; the same applies for grazing where methane emissions from the excreta are likely to be very low (Johnson *et al.*, 1997). Reports in the literature on the effects of different diet types on CH_4 emission from the slurry are very rare and most data on CH_4 emission from slurry were obtained so far from biogas plant research. For biodigester, the CH_4 production mostly is expressed per m^2 slurry surface or m^3 slurry volume. However, CH_4 production per m^2 slurry surface can be highly variable, since it rather depends on the amount of biomass underneath the surface. Safely and Westermann (1988) for instance measured between 0.05 and 0.50 m^3 CH_4 per m^2 slurry surface per day on the same farm but at two different dates.

Ellgaard (2001) reported that typical extents of OM degradation in slurry range between 500 and 600 g kg^{-1}, while Safely (1989) assumed that almost all of the non-lignin OM is fermented in anaerobic lagoons. In the present study, OM degradation in the slurry was found to range between 290 and 500 g kg^{-1}, where values were lowest in the slurry from the cows fed the oat hulls diet.

The results of the present study were in the range as the ones observed by Külling *et al.* (2002). However, recalculating the present results on CH_4 emission during 14 weeks of slurry storage from kg^{-1} to m^{-3} slurry results in 4.2 to 7.5 kg m^{-3} CH_4, which is up to two times higher than the 3.6 kg m^{-3} described by Clemens *et al.* (2004). The noted increase and decrease of CH_4 during slurry storage has also been observed in Møller *et al.* (2004) and Külling *et al.* (2002). One reason for that could be the decrease of available nutrients with time or the decline in water content of the slurry.

In the present study, having almost anaerobic conditions in the slurries, the CH_4 emission from the slurries made up between 16.0 and 21.9% of the total (enteric & slurry) CH_4 emission of the animal. This proportion is in the range reported by Külling *et al.* (2002), where between 9.5 and 27.3% of total CH_4 emission originated from slurry storage, when feeding diets supplemented with different fatty acids to dairy cows.

Külling *et al.* (2002) observed that the CH_4-suppressing effect of a medium-chain fatty acid (lauric acid) in the rumen could be partly compensated by a corresponding increase in CH_4 emission from slurry storage. This was explained by the higher excretion of degradable fiber in the slurry of these cows. However, the

present study demonstrated that this compensation is not a general phenomenon of a low fiber digestibility as it depends strongly on the potential degradability of the fiber excreted with the feces. The potential degradability was presumably high in the study of Külling *et al.* (2002) as the lower fiber degradation resulted from microbial inhibition and not from the physico-chemical properties of the fiber. Accordingly, the reduction in CH_4 emission by the strongly lignified diet (oat hulls diet) during enteric fermentation was not compensated in the slurry in our study because fermentable OM content was low also in the slurry. The other five diets did not significantly differ in their effect on methanogenesis in the slurry, even though a significant difference existed in fiber degradation among the diets. Both the experiment by Külling *et al.* (2002) and the present study indicate that the mechanisms of anaerobic methanogenesis in the slurry and in the animal are very similar, although the formation of CH_4 in the slurry takes places much slower. Our measurements confirm that it is necessary to measure methane from slurry over a sufficiently long period, as stated by Külling *et al.* (2002), in order to be able to include the major phase of methanogenesis. Since the slurry of dairy cows and beef cattle kept in barn is often stored in ponds over extended periods of time, particularly in wintertime, this is not a negligible source of CH_4 in animal production.

In the present study, CH_4 emitted from slurry was positively correlated with CH_4 emission from the animal, but the correlation was not very close. A high variation of the CH_4 emission from slurry replicates probably prohibited a closer relationship. Methane was emitted at irregular intervals from the slurry, and the accuracy of determining the actual emission might increase when extending the measurement period to more than 9 h, and/or when investigating a larger number of replicates. Although contributing to individual variation among cows within dietary treatment, certain dietary differences may have additionally developed since they resulted in systematically differing amounts and contents of DM of the slurries. Another factor contributing to the variation in CH_4 emission was the individual variation among cows (within dietary treatment). It is questionable whether or not the variation should be reduced by standardizing slurry DM contents before storage as the DM content is a real variable in farm practice, too.

Although the amount of degraded carbohydrate had similar effects on CH_4 emission both in the animal and in overall system including slurry storage, there were still some differences between enteric and total methanogenesis which might even be higher when also the forage-to-concentrate ratio is varied.

4.4. Nitrogen and ammonia-N loss from the slurry

Nitrogen intake and total excretion of the cows was similar across diets, but there were significant shifts in the proportion of the easily-volatile urine N of total N, particularly concerning the oat hulls diet as opposed to all other diets. This was probably the result of differences in N incorporation into microbial biomass due

to the different supply of fermentable OM needed to remove rumen fluid ammonia (Valk, 1994). Accordingly, there was a weak trend for the slurry of the cows fed the oat hulls diet to express proportionately higher gaseous N losses during storage, equalizing initial differences in ammonia and total slurry N contents. However, the difference to the other diets was lower than expected (Külling *et al.*, 2001 and 2003). This may have been the consequence of the high initial and final DM content of this type of slurry and of the numerically lower N intake of these cows resulting in a low total N content of the corresponding slurry. Much greater differences in gaseous N losses can be expected from diets providing N far in excess of the animal's requirements (Külling *et al.*, 2001, 2003).

5. Conclusion

The first aim of the study was to investigate if there is a compensatory increase in CH_4 emission from slurry in cases of a higher fiber excretion of ruminants. This was not the case in the present study. The diet rich in lignified diet reduced both fiber digestibility and methanogenesis in the animal, and the same was found during slurry storage. Also soluble carbohydrates reduced the fiber digestibility in the animal, but the increased fiber content in the manure was not enough to enhance methane emission from the slurry significantly. Secondly, the results showed that a detailed carbohydrate analysis in feed is required in order to satisfactorily predict CH_4 emissions both from the animal's digestion and from manure storage and to identify concentrates and complete diets with low inherent methane production potential. Accordingly, starchy concentrates should be preferred to those rich in sugar. In equations available so far, the methanogenic potential of sugars and the CH_4-preventing action of lignin is not sufficiently accounted for. This could be shown even when adjusting data for DM intake. Thirdly, the study confirmed that slurry is a significant source of CH_4 emissions also in ruminants as it contributes up to 22% of total emission, depending on the length of the slurry storage period. The variability in CH_4 emission from the slurry, however, was much higher than that of enteric CH_4 emission, which makes predictions more difficult. Data finally show that, focusing on CH_4 mitigation through a strategic use of carbohydrates in the concentrate, would have only limited side-effect on gaseous N losses from slurry storage.

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