Changes in methane release from organic matter passing through the digestive tract of horses

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ABSTRACT: Using the tests of methanogenic activity (TMA) changes in methane yield (Y_{CH4}) and anaerobic degradability (D_c) of organic matter of feeds and excrements were studied in an experimental group of six horses while complete analytical methods were applied (N-compound matters, proteins, non-protein N-compound matters, fat, nitrogen-free extract, ash, crude fibre, organic matter, NDF, ADF, hemicelluloses, cellulose, lignin and chemical oxygen demand COD) and the material balance was determined. The horses utilised 48.8% of organic matter of feeds in dry matter while the daily weight of droppings was 21 kg with 5.20% of dry matter and 4 kg of urine with 7% of organic matters. It is important that the theoretical methane yield per 24 hours corresponding to the organic matter of ingested feeds which was transferred to excrements is 1.771 m^3 CH_4 at 0°C and 1 013.25 hPa while the actual daily methane yield of droppings is 1.739 m^3 CH_4 at 0°C and 1 013.25 hPa, i.e. practically identical, because the yield from urine organic matters was not included in the actual daily methane yield. Because the anaerobic degradability of the used feed mixture and horse droppings is practically identical, it is obvious that besides the enteric fermentation according to the reaction $CO_2 + 4\ H_2 \rightarrow CH_4 + 2\ H_2O$ by hydrogenotrophic methanogens no classical anaerobic digestion takes place in the digestive tract of horses; it means that the horse breeding sector is not a factor contaminating the atmosphere by methane.

Keywords: horses; feeds; horse droppings; organic matter; methane yield; anaerobic degradability

Methane is one of the most important greenhouse gases, the production of which has to be reduced in accordance with the Kyoto Protocol. From the aspect of global climate changes expressed by the value of global warming potential (GWP) per 100 years it is 21 times more noxious that CO_2 (Schachermayer et al., 1999). It is produced in the process of anaerobic digestion that takes place at several phases in the presence of various groups of microorganisms in organic matter in an anaerobic medium (Baresi et al., 1978). It exists spontaneously in many forms in the natural environment (Garcia et al., 2000). Four phases can be distinguished: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Gujer and Zehnder, 1983). The processes of this biochemical conversion are completely interconnected with each other in the natural course of the process, so there is no accumulation of intermediary products of the system (Kaseng et al., 1992). The whole process is governed by microorganisms of two kingdoms: Bacteria and Archaea (Dugba and Zhang, 1999). In the hydrolytic phase organic matter is depolymerised by the effect of hydrolytic enzymes (Kaseng et al., 1992), in the acidogenesis phase products of hydrolysis are transformed to lower organic acids and CO_2,

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H_2 and lower alcohols (Kalyuzhnyi et al., 2000). It is the fastest reaction of anaerobic transformation (Schink, 1997). In the acetogenesis phase higher fatty acids are transformed to anions of acetic acid (partly also of formic acid), CO_2 and H_2 (Salminen et al., 2000). The methanogenesis phase is the production of methane from H_2, CO_2 and anions of acetic acid, partly also from formates, methanol and methylamines (Hwang et al., 2001).

The main sources of methane in the natural environment are places of anaerobic degradation of organic matter. These are peat bogs (Juottonen et al., 2006), wetlands, rice fields (Penning and Conrad, 2007) and mud filled water recipients (Umemura et al., 2006), household solid waste dumping sites containing a biodegradable component (Farquhar and Rovers, 1973) and, in general, all dumps of organic waste. Ecologists consider animals, mainly ruminants (Gijzen, 1998), as an important source of CH_4 production. The hydrolysis of feed cellulose and hemicelluloses in their digestive tract results in the production of large amounts of H_2 and CO_2. But hydrogenotrophic methanogens in this medium contribute to a significant reduction in the volume of produced gases at a 1:5 ratio according to the equation:

$$CO_2 + 4\ H_2 \rightarrow CH_4 + 2\ H_2O$$

If active oxygen penetrates into the digestive system, e.g. in the form of fresh green forage with still active photosynthetic processes, it may cause the intoxication of hydrogenotrophs or even fatal tympanites requiring a veterinary intervention. In normal conditions the above equation describes so called enteric fermentation, which substantially contributes to emissions of greenhouse gases. They are classified among anthropogenic emissions (Straka et al., 2003).

Research on the contamination of atmosphere by greenhouse gases produced by farm animals is aimed at the development of prediction models of CH_4 production which simulate dry matter intake (DMI), metabolisable energy intake, intake of neutral detergent fibre NDF and acid detergent fibre ADF, fats expressed as ether extract and lignin content in feed rations for cattle and dairy cows separately (Kamalak et al., 2005; Třináctý et al., 2005a,b; Ellis et al., 2007). Attention is paid to CH_4 production in different species of farm animals. Muenger and Kreuzer (2008) studied differences in the potential production of CH_4 in dairy breeds Holstein, Jersey and Simmental. No breed differences were observed. Dong et al. (2006) measured methane production in pigs, Hegarty et al. (2007) measured it in breeding bulls, and Alcock and Hegarty (2006) in sheep.

Measurement accuracy is a problem when measuring the potential methane production because it is complicated by a different rate of the above-mentioned enteric fermentation, specificity of the process of digestion of each individual and perhaps by the stress of animals caused by measurements in respiratory chambers with harnesses for the fixation of animals and apparatuses; the above-mentioned authors reported that the methane production related to the unit of feed organic dry matter (DM) or of residual feed (RFI) and/or of NDF and ADF content was not stabilised but it varied slightly during experiments conducted in stationary conditions.

Many papers describe the determination of degradability of nutrients from feeds at different phenological stages in fresh and ensiled matter (Čerešňáková et al., 2005, 2007; Niwinska et al., 2005; Jančík et al., 2008). NDF degradability was studied by an *in sacco* method. The comparison of ruminal degradation of lucerne, clover and grasses showed the highest value in legumes (62.9–67.1%) and the lowest value in grasses (49.5–51.5%). Studying ruminal degradation by an *in sacco* method Jančík et al. (2008) reported the existence of a high correlation between digestible neutral detergent fibre (DNDF) and acid detergent lignin (ADL) ($P < 0.05$, $r = -0.87$), which is a reliable parameter for an estimation of the content of indigestible neutral detergent fibre (INDF).

Kamalak et al. (2005) investigated the degradation of wheat straw, barley straw, lucerne and maize silage by *in vitro* and *in situ* methods in nylon bags with measurement of gas (CO_2) production and description of the process kinetics.

Contrary to direct measurements of methane production in animals, the objective of our study was to determine changes in the methane yield Y_{CH4} of organic matter of inputs and outputs (feeds and excrements) by tests of methanogenic activity (TMA), to compare the measured values with the theoretical yield of methane $Y_{CH4\ m\ theor}$ per unit weight of the substrate of these organic matters and to find differences in the anaerobic degradability of feeds and horse droppings. Based on the results and material balance a conclusion will be drawn whether the organic matter markedly changed its methane yield after passage through the digestive tract of horses and changes in it.

MATERIAL AND METHODS

An experiment was conducted with a group of six horses and it lasted for six days. In the first three days the animals were fed to adjust the feed ration in a preparatory phase, the own experiment with the collection of all horse droppings in fresh condition and their weighing lasted for another three days. Feeds were oats, hay and straw; hay was administered 3 times a day and oats twice a day. Table 1 shows the characteristics of the experimental group of horses, feed ration and weight of horse droppings.

Feed mixture of oats, hay and straw at a ratio 1:7.88:1.15 was administered at an amount 10.4 to 15.8 kg according to weight in accordance with the common norm.

NDF and ADF were determined in feeds and excrements (ADF contains lignin, cellulose and mineral fraction while NDF contains hemicelluloses in addition. The content of hemicelluloses is determined by subtraction of ADF from NDF, lignin content is determined from ADF before and after oxidation with $KMnO_4$. After ashing the ADF residue in a muffle furnace, the weight of mineral fraction is determined by weighing the residue, after its subtraction from the ADF residue the content of cellulose in a sample is determined). The methodology was described by Van Soest (1963), and in our study we used the modification according to López et al. (1992), which is practically identical with Czech methodology of the Central Institute for Supervising and Testing in Agriculture. Current routine analytical methods were used to determine water, N-matters (Kjeldahl), proteins (Barnstein), fat (hexane extraction in Soxhlet), ash (550°C), dry matter (105°C), crude fibre (Henneberg – Stohmann) and to calculate amides and nitrogen-free extract.

Chemical oxygen demand COD was determined in all tested materials (Sedláček, 1978), and based on it, theoretical yield of methane was calculated and expressed as the weight amount of methane per unit weight of substrate according to Straka et al. (2003).

$$Y_{CH4\ m\ theor} = 0.25\ COD \qquad (g/g)$$

Because the materials of feeds and excrements did not have a negligible amount of nitrogen and sulphur, the theoretical yield of methane was corrected by the subtraction of chemical demand of oxygen consumed for a reduction of nitrogen and sulphur according to the modified equation

$$Y_{CH4\ m\ theor} = 0.25\ (COD - N - S) \qquad (g/g)$$
$(CH_4$, substrate)

where:

N = oxygen equivalent of nitrate and nitrite nitrogen = 2.86 $(NO_2{}' - N + NO_3{}' - N)$ (g/g) $(O_2$, COD)

S = oxygen equivalent of sulphur = $2(S_{tot})$ (g/g) $(O_2$, COD)

Determined coefficients are of empirical character. Straka et al. (2003) reported the details.

The substrate production of methane V_{CH4S} (the volume of produced methane (V_{CH4c}) after subtraction of endogenous production of methane (V_{CH4e}) by the inocula) was determined by an

Table 1. Characteristics of experimental group of horses, feed ration and weight of horse droppings

Name of the horse	Weight (kg)	Breed	Age (year)	Load	Weight of droppings (kg)		
					total	per day	1 excrement
Nero	650	CT	9	light	64.70	21.57	1.66
Miss	534	CT	8	light	61.62	20.54	1.19
Calledon	634	CT	7	light	69.82	23.27	1.49
Korrika	495	A1/1	17	light	58.80	19.60	1.18
Coral	713	CT	9	light	64.65	21.55	1.80
Labello	484	A1/1	13	light	58.00	19.33	1.07

CT = Czech warm-blooded horse; A1/1 = English Thoroughbred

Oxi Top Control Merck measuring system, with measuring heads with piezoelectric pressure sensors with infrared interface by means of which it is possible to communicate with the controller OC 100 or OC 110, which may administer up to 100 measuring heads. Documentation is done by the ACHAT OC programme in connection with PC or TD 100 thermoprinter. Measuring heads will store up to 360 data records in their memory that may be graphically represented in the controller.

The calculation is based on this equation of state:

$$n = p \times V/RT$$

where:
n = number of gas moles
V = volume (ml)
p = pressure (hPa)
T = temperature (°K)
R = gas constant 8.134 J/mol°K

and the number of CO_2 and CH_4 moles in the gaseous phase of fermentation vessels is calculated:

$$n_{CO2\,g\,CH4} = (\Delta p \times Vg/RT) \times 10^{-4}$$

$$\Delta p = p_1 - p_0$$

where:
p_0 = initial pressure

Fermentation at 35°C and continuous agitation of vessels in a thermostat lasts for 60 days, the pressure range of measuring heads is 500–1 350 kPa and the time interval of measuring pressure changes is 4.5 min. Anaerobic fermentation is terminated by the injection of 1 ml of 19% HCl with a syringe through the rubber closure of the vessel to the substrate. As a result of acidification CO_2 is displaced from the liquid phase of the fermentation vessel. The process is terminated after 4 hours. The number of CO_2 moles is calculated from the liquid phase:

$$n_{CO2\,l} = ((p_2\,(V_g - V_{HCl}) - p_1 \times V_g)/RT) \times 10^{-4}$$

The injection of 1 ml of 30% KOH into the rubber container in the second tube of the fermentation vessel follows. The sorption of CO_2 from the gaseous phase of the vessel is terminated after 24 hours and the total number of CO_2 moles in gaseous and liquid phases is calculated from a drop in the pressure in the vessel:

$$n_{CO2\,l,\,CO2\,g} = ((p_3(V_g - V_{HCl} - V_{KOH}) - p_2(V_g - V_{HCl}))/RT) \times 10^{-4}$$

where:
Δp = difference in pressures (hPa)
V_g = the volume of the gas space of fermentation vessel (ml)
p_1 = gas pressure before HCl application (hPa)
p_2 = gas pressure before KOH application (hPa)
p_3 = gas pressure after KOH application (hPa)
R = gas constant = 8.134 J/mol°K
T = absolute temperature = 273.15 + X°C
V_{HCl} = the volume of added HCl (ml)
V_{KOH} = the volume of added KOH (ml)

Based on the results, it is easy to calculate the number of CO_2 moles in the gaseous phase and by subtraction from $n_{CO2\,g\,CH4}$ the number of moles of produced methane:

$$n_{CH4} = (n_{CO2\,g\,CH4} + n_{CO2\,l}) - n_{CO2\,l\,CO2\,g}$$

The total number of moles of the gases of transported carbon:

$$n_{CO2\,g\,CH4} + n_{CO2\,l} = n_{total}$$

Baumann's solution A + B in deionised water of pH = 7.0 is used as a liquid medium (Süssmuth et al., 1999).

The standard addition of inoculum corresponds roughly to an amount of 0.3% by volume (aqueous sludge from the anaerobic tank of the fermenter). Instead of Baumann's solution it is possible to use a ready-made nutrient salt of the MERCK company for this system.

Work details were described in our papers published in the last years (Kolář et al., 2003, 2006), the operation of the Oxi Top Control measuring system was described in detail by Süssmuth et al. (1999).

Methane yield was calculated from the substrate production of methane V_{CH4S} by division by the initial amount of added substrate:

$$Y_{CH4g} = \frac{(V_{CH4C} - V_{CH4e})}{S} = \frac{V_{CH4S}}{S} \quad (l/g)$$

where:
V_{CH4C} = methane yield of C-source
V_{CH4e} = methane yield of added inoculum
S = quantity of substrate at the beginning (g)

In addition, the anaerobic degradability of feeds and excrements was determined. The method of evaluation based on organic carbon was used for this purpose. Degradability is given by the equation:

$$D_c = \frac{C_g}{C_s} \times 100 \qquad (\%)$$

where:

C_g = the content of carbon in the gaseous phase at the end of the test of methanogenic activity and is calculated from this equation:

$$C_g = \frac{12\ p \times V_{CH4S}}{RT}$$

where:

p = pressure
V_{CH4S} = substrate production of methane
R = gas constant
T = temperature (°K)
C_s = the content of organic carbon of the substrate added at the beginning of the test

Lord's test and other methods suitable for few-element sets and based on the range R of parallel determinations (Sachs, 1974) were used for the mathematical and statistical evaluation of analytical results including the computation of the interval of reliability.

The value of COD for the oxidation of organic matter more or less approximates the theoretical consumption of oxygen that is defined for a material containing carbon (a), hydrogen (b) and oxygen (c) in this way:

$$TOD = \frac{(4a + b - 2c) \times 8}{M_r} \qquad (g/g)$$

where:

M_r = relative molecular weight

TOD is a theoretical consumption of oxygen for oxidation of any organic matter, expressed by the quantity of oxygen in grams, necessary for total oxidation of 1 gram of organic matter according stoichiometry. The degree of chemical oxidation of organic matters COD is then compared with TOD.

Table 2. The analysis of average samples of feeds and pre-dried droppings and their chemical oxygen demand COD (g O_2/g DM)

	Oats	Hay	Straw	Feed mixture of oats, hay and straw at a ratio used for feeding	Droppings
Water (%)	12.14	15.07	14.94	14.75	75.21
N-matters (%)	11.93	8.51	3.65	8.36	2.07
Proteins (%)	8.48	6.48	2.55	6.28	–
Non-proteinaceous N-matters (%)	3.45	2.03	1.10	2.07	–
Sulphur (%)	0.19	0.11	0.22	0.14	0.12
Lipids (%)	8.03	0.90	1.54	1.67	4.74
Nitrogen-free extract (%)	53.75	39.32	31.06	39.94	3.23
Ash (%)	2.21	5.02	4.89	4.83	2.53
Dry matter (%)	87.86	84.93	85.06	85.23	24.79
Fibre (%)	11.94	31.05	43.92	30.43	13.82
Organic matter (in DM) (%)	97.49	89.23	90.73	90.20	92.94
NDF (%)	25.93	64.25	72.50	61.24	13.15
ADF (%)	9.05	32.51	48.87	31.81	9.02
Hemicelluloses (%)	16.88	31.74	23.63	29.44	4.13
Cellulose (%)	6.10	23.45	36.66	23.04	7.82
Lignin (%)	0.74	3.91	7.32	3.93	1.20
COD (g O_2/g DM)	1.23	0.97	0.94	0.99	0.96

Besides, in the course of two-hour boiling of dichromate with sulphuric acid and sample autocatalytic processes cause a substantial reduction of Cr^{VI} to Cr^{III}; nevertheless, COD is practically always lower than TOD. It is explained by different kinetics of oxidation as the first-order reaction – the rate constant of oxidation fluctuates in the interval of up to three orders in different materials. All examined materials contain polysaccharides, fat and lignin as prevailing matter, and therefore COD is quite a satisfactory value to determine the theoretical yield of methane in this case. Because TOD of fats (2.92) is approximately 3.4 times higher than that of polysaccharides (0.85) (starches, hemicelluloses and cellulose), the content of fats in the sample obviously plays an important role in the value of COD. TOD of lignin is also relatively high (2.20) and markedly increases COD, but it is only weakly degradable in the process of anaerobic digestion, so it influences the methane yield in a negligible way. The empirical formula for the recalculation of COD to $Y_{CH4\ m\ theor}$ considers this fact.

RESULTS AND DISCUSSION

The results in Table 2 document that the COD of horse droppings is surprisingly high and its value approximately corresponds to the COD of hay used for feeding and is slightly higher than the COD of feeding straw. So there is no difference in the theoretical methane yield of horse droppings and used feed (Table 3), and the real methane yield Y_{CH4g} of the feed mixture of oats, hay and straw at a ratio used for feeding is practically identical with the methane yield of horse droppings. Anaerobic degradability D_c of this mixture is slightly higher than anaerobic degradability of droppings but the difference (2.24%) is negligible.

Comparing the feeds and excrements, the two values of TMA, methane yield Y_{CH4g} and anaerobic degradability D_c, explicitly prove that no anaerobic digestion took place in the digestive tract of horses in this experiment, that methane is probably produced there only by a reduction in carbon dioxide by hydrogenotrophic methanogens and that horse droppings may be an ex-

Table 3. Results of the tests of methanogenic activity (TMA) of feeds and droppings in dry matter (Interval of reliability of the average for resultant values in relation to material balance was calculated for a significance level α = 0.05)

	Oats	Hay	Straw	Feed mixture of oats, hay and straw at a ratio used for feeding 1:7.88:1.15	Droppings
Theoretical yield of methane $Y_{CH4\ m\ theor}$ (g/g)	0.31	0.24	0.23	0.246	0.240
Corrected theoretical yield of methane (g/g)	0.31 ± 0.01	0.24 ± 0.02	0.23 ± 0.01	0.24 ± 0.02	0.24 ± 0.01
Substrate production of methane V_{CH4S} (l)	0.22	0.63	0.60	0.586	0.700
Methane yield Y_{CH4g} (l/g)	0.48 ± 0.05	0.32 ± 0.03	0.29 ± 0.02	0.33 ± 0.03	0.36 ± 0.04
C content in gaseous phase at the end of TMA C_g (g)	0.058	0.282	0.254	0.257	0.254
Organic C content in substrate at the beginning of TMA C_s (g)	0.203	0.851	0.835	0.785	0.842
Anaerobic degradability D_c (%)	28.93 ± 1.52	33.17 ± 3.30	30.51 ± 3.00	32.48 ± 2.93	30.24 ± 2.68

Table 4. Material balance of the experiment

Feeds	(kg)	Excrements	(kg)
Daily feed intake (DFI)	13	daily weight of droppings (DWD)	21
Dry matter of DFI	11.07	dry matter of DWD	5.20
Organic matter in dry matter of DFI	9.98	organic matter in dry matter of DWD	4.83
		daily weight of urine (DWU)	4
		organic matter in DWU at 7% of organic matters	0.28
Transfer from DFI to DWD and DWU			
Organic matter in dry matter of excrements (urine + droppings) in % of organic matter of feed		(4.83 + 0.28)/9.98 × 100 = 51.2%	
Chemical oxygen demand corresponding to transferred organic matter in dry matter		5 058.7 g O_2	
Theoretical methane yield corresponding to organic matter of DFI in dry matter that was transferred to excrements		1 264.7 g CH_4 i.e. 79 moles of CH_4 = 1.771 m^3 CH_4 at 0°C and 1 013.25 hPa	
Actual daily methane yield of droppings		1.739 m^3 CH_4 at 0°C and 1 013.25 hPa	

cellent material not only as a fertiliser but also as a raw material for biogas production. It is to remind that this method of their use would markedly diminish the value of horse dung as valuable organic manure.

The above results are also confirmed by the value of the corrected theoretical yield of methane $Y_{CH4\ m\ theor.\ corr.}$ which is practically identical in excrements and feeds at the used ratio (Table 3).

Finally, it is to explain why the values of theoretical methane yield and corrected values of theoretical methane yield in Table 3 are identical.

Because the content of total sulphur was 0.11% in feeding hay and twice higher in straw, and also the S content in droppings is low, the correction of theoretical methane yield would be indicated at the third decimal place. The correction for nitrate nitrogen would be identifiable at a further place. As the accuracy of COD determination allows to give results only with 2 decimal places, the corrected theoretical methane yield in Table 3 is identical to that before correction.

If TMA confirmed that methanogenesis in the digestive tract of horses (except for a CO_2 reduction by hydrogen to methane) was quite negligible, we want to accentuate for comparison that e.g. a mixture of pig slurry and sludge from waste water treatment plants lowers anaerobic degradability after anaerobic digestion in a fermenter during mesophilic digestion about 2× to 4×.

Lipids are known to be degraded to the greatest extent during anaerobic digestion while lignin degradation is the lowest even though we speak about cellulose fermentation. Table 2 documents that mainly the content of nitrogen-free extract dropped. Taking into account that NFE is composed of saccharides and easily hydrolysable saccharides that are used by animals for energy acquisition, it is not a surprise. But a minimum decrease in lipids was quite surprising; it proves again that the methanogenic process in the digestive tract of horse was hardly observable in this experiment.

Daily weight of droppings (DHS) in comparison with the daily weight of ingested feeds (DPK) is higher owing to different dry matter. If the organic matter is metabolised in animals, we would have to find a significant difference between the theoretical yield of methane, methane yield (Y_{CH4g}) and anaerobic decomposability (Dc) of excrements and feeds.

The material balance in Table 4 confirms the above findings. The actual daily methane yield of horse droppings (without measuring the CH_4 yield in urine) is only slightly lower than the theoretical value, corresponding to the organic matter transferred from feeds to excrements. No anaerobic digestion of this matter practically took place.

It is to conclude that the horse breeding sector is not among factors contaminating the atmosphere by methane. It is also confirmed by the high anaerobic degradability of horse droppings which differs by 2% only from the initial organic matter of feed mixture, which can be considered as a small experimental error.

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