Anaerobic degradability of organic matter of cattle faeces and a possibility of its utilization

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ABSTRACT: The content of organic matter labile fractions is negligible in cattle faeces but the content of the anaerobically degradable fraction on the boundary of organic matter lability and stability is analytically utilizable and different. It depends not only on feed quality and quantity but also on all factors of enteric fermentation and processes determining the digestion of ruminants. In the present paper we attempted to describe the digestive tract of ruminants as an anaerobic bioreactor and to calculate its mass balance so that the measurement of a difference in the anaerobic degradability of feeds and faeces at steady state could be theoretically demonstrated as a multipurpose research method not bothering the animals. The first results in eight variants proved that feed quality and other factors influencing processes in the digestive tract of ruminants could be monitored in this way. Obviously, this method does not replace the present methods used for feed digestibility determination.

Keywords: degradability of phytomass; anaerobic bioreactor; organic matter; difference in anaerobic degradability of feed and cattle faeces; possibility of utilization

Any organic matter of natural origin has its labile and stable fractions (Kolář et al., 2009a). In both these groups of fractions there are anaerobically degradable fractions to which all labile fractions also belong (Kolář et al., 2010). Mainly ecologists who fear that the atmosphere may be contaminated by greenhouse gases produced by animal farms are interested in the anaerobic degradability of organic matter (Gijzen, 1998; Alcock and Hegarty, 2006; Dong et al., 2006; Hegarty et al., 2007; Muenger and Kreuzer, 2008). Our team studied the yield of methane from organic matter passing through the digestive tract of horses and we found out that such a fear was little reasonable in most cases (Kolář et al., 2009b). The main sources of methane in the atmosphere are wetlands, landfills, peat bogs and paddy fields (Umemura et al., 2006; Penning and Conrad, 2007). In general, organic matter degradability is studied by specialists in animal nutrition, by forage specialists, compost producers and specialists in community hygiene and biogas production. Many papers are aimed at determining the degradability of fresh and ensiled organic matter (Čerešnáková et al., 2005; Niwinska et al., 2005; Čerešnáková et al., 2007). Ruminal degradation is studied by *in sacco* methods, the content of indigestible neutral detergent fibre (INDF) is estimated (Jančík et al., 2008) as well as the relations of dry matter intake (DMI), metabolizable energy intake, neutral detergent fibre (NDF), acid detergent fibre (ADF), fats and lignin in the feed ration for cattle and especially for dairy cows (Třináctý et al.,

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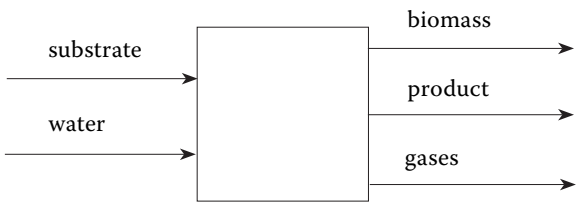
2005a,b), degradation of feed organic matter by *in vitro* and *in situ* nylon-bag methods with measurement of gas production and description of the process kinetics (Kamalak et al., 2005). Degradability of organic matter in other disciplines is investigated particularly from the aspect of resistance in acid hydrolysis (Rovira and Vallejo, 2002; Shirato and Yokozava, 2006) or in oxidation (Blair et al., 1995; Chan et al., 2001). Because it determines the reaction time of organic matter transformations, it plays an important role in many practical applications (Frelich et al., 2009; Jančík et al., 2009; Hanuš et al., 2010; Jurajda et al., 2010; Váradyová et al., 2010).

In an anaerobic space in the presence of hydrolytic enzymes the anaerobically degradable fraction of any organic matter provides its lipids, proteins and polysaccharides in the acidogenic phase of a set of processes for transformation to aliphatic carboxylic acids and simple sugars from which CO_2 and H_2 are produced by the activity of acidogenic microorganisms. Both main substrates are then converted to methane by acetotrophic and hydrogenotrophic methanogens. In ruminant digestion the sequence of reactions is identical (Gijzen, 1998) and it makes us formulate a hypothesis that in the system conception of steady state the digestive tract of a ruminant can be considered as an anaerobic bioreactor and therefore its calculation can be performed. We ask a question whether it is possible to monitor the course of the bioreaction on the basis of the elemental balance of carbon forms and what measurement is necessary on condition that biomass and product are generated. The product is taken to mean more or less utilized C sources at the output, i.e. faeces.

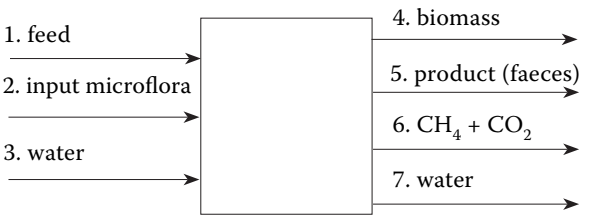
MATERIAL AND METHODS

The problem is solved by calculating the mass balance of a bioreactor. The theory of balancing was clearly described in Treybal (1968), Coulson and Richardson (1977) and Benitez (2002).

(a) Balance scheme of a bioreactor



(b) Considered materials of a bioreaction



After the integral balance period has elapsed, the bioreactor contains biomass, faeces, water, utilized and unutilized feed, gases.

The particular flows are materials participating in the bioreaction, not the own process flows. The fictitious reaction flows are not given because it is assumed that the problem can be solved on the basis of the mass balance of elements.

The elements 1-C, 2-H, 3-N and 4-O are defined as components to be balanced.

The matrix of the assignment has the following form (Figure 1)

The matrix notation of the system of balance equations (Figure 2)

If the matrix of coefficients is known, the system contains seven unknown masses for 4 independent balance equations of element masses. From the balance 4 data on quantity can be determined and 3 data on the quantity of materials must be determined by measurement. Because the measurement of m_6 (biogas production in digestive tract) is reflected in a change in the form of C in m_1 and m_5, this measurement can be completely excluded. The measurement of water quantity in the whole system is problematic because it is both at the input and at the output, and therefore m_3 and m_7 can be excluded by the monitoring of only dry matter m_1 and m_5. The values of m_2 and m_4 are a part of m_1 and m_5. The accumulated value of m_4 (an increase in animal biomass) is negligible in the balance period. It is to state that the course of the bioreaction in the digestive tract of ruminants can be described reliably only by determination of changes in carbon quality and quantity in the dry matter of component m_1 and m_5 mass, i.e. feed and faeces in the given balance period.

To determine the carbon quantity the quantity of oxygen necessary for its chemical oxidation, i.e. COD (chemical oxygen demand), is generally used as the unit of measure. The quality of carbon is assessed according to anaerobic degradability and its

j →	1	2	3	4	5	6	7
↓ i m_j/kg	?	?	?	?	?	?	?
1–C	$X_{1,1}$	$X_{1,2}$	0	$X_{1,4}$	$X_{1,5}$	$X_{1,6}$	0
2–H	$X_{2,1}$	$X_{2,2}$	$X_{2,3}$	$X_{2,4}$	$X_{2,5}$	$X_{2,6}$	$X_{2,7}$
3–N	$X_{3,1}$	$X_{3,2}$	0	$X_{3,4}$	$X_{3,5}$	0	0
4–O	$X_{4,1}$	$X_{4,2}$	$X_{4,3}$	$X_{4,4}$	$X_{4,5}$	$X_{4,6}$	$X_{4,7}$

Figure 1. The matrix of the assignment

$$\begin{Bmatrix} X1,1 & X1,2 & 0 & X1,4 & X1,5 & X1,6 & 0 \\ X2,1 & X2,2 & X2,3 & X2,4 & X2,5 & X2,6 & X2,7 \\ X3,1 & X3,2 & 0 & X3,4 & X3,5 & 0 & 0 \\ X4,1 & X4,2 & X4,3 & X4,4 & X4,5 & X4,6 & X4,7 \end{Bmatrix} \begin{Bmatrix} -m1 \\ -m2 \\ -m3 \\ m4 \\ m5 \\ m6 \\ m7 \end{Bmatrix} = \begin{Bmatrix} 0 \\ 0 \\ 0 \\ 0 \end{Bmatrix}$$

Figure 2. The matrix notation of the system of balance equations

change in components m_1 and m_5, so it is not necessary to measure the masses of these components.

The experimental animals represented 3 sets: set A with the number of animals n = 4, Šumava locality, South Bohemia, set B with n = 6, Kadaň locality, central Poohří area and set C with n = 6, Prachatice locality, South Bohemia. The sets were selected randomly for these preliminary experiments while the age, health status and other important factors were not taken into account. *Ad libitum* feeding through grazing was used to ensure the stationary state. Feed quality differed in the quality of fodder herbage, and with regard to the objective of this research it was not assessed from the fodder specialist's aspect but it was assessed on the basis of hydrolytic degradability by the Shirato and Yokozawa (2006) method and by two different methods of oxidative degradability determination according to Blair et al. (1995) and Chan et al. (2001).

In the second part of experiments the quality of feeds and faeces was investigated only from the aspect of anaerobic degradability and its changes in Red Pied cattle in Šumava, in the localities Vlčí Jámy (D), Velhartice (E), Buk (F), Těšov (G) and Rychnov (H).

Chemical oxygen demand (COD) was determined in all tested materials (Horvitz and Latimer, 2005) and based on it, theoretical yield of methane was calculated and expressed as the mass amount of methane per unit mass of substrate according to Szendrey (1983).

$$Y_{CH4mtheor} = 0.25\ COD \quad (g/g)$$

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

$$Y_{CH4mtheor} = 0.25\ (COD - \underline{N} - \underline{S}) \quad (g/g)$$
$$(CH_4, \text{substrate})$$

where:

$\underline{N}$ = oxygen equivalent of nitrate and nitrite nitrogen:

$\underline{N} = O_2\ eq \times (NO_2^- + NO_3^-)$

$\underline{N} = 2.86\ (NO_2' - N + NO_3' - N)$ (g/g) (O_2, COD)

$\underline{S}$ = oxygen equivalent of sulphur:

$\underline{S} = O_2\ eq \times S_{tot}$

$\underline{S} = 2\ (S_{tot})$ (g/g) (O_2, COD)

Determined coefficients are of empirical character (Szendrey, 1983).

The substrate production of methane V_{CH4S} (the volume of produced methane (V_{CH4c}) after the subtraction of endogenous production of methane (V_{CH4e}) by the inocula) was determined by an Oxi Top Control Merck measuring system, consisting of measuring heads with piezoelectric pressure sensors with the 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 represented graphically 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:

$$n_{CO_2gCH_4} = (\Delta p \times Vg/RT) \times 10^{-4}$$
$$\Delta p = p_1 - p_0$$

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:

$$n_{CO2l} = \{(p_2\ (V_g - V_{HCl}) - p_1 \times V_g)/RT\} \times 10^{-4}$$

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:

$$n_{CO_2l,CO_2\,g} = \{(p_3\ (V_g - V_{HCl} - V_{KOH}) - p_2\ (V_g - V_{HCl}))/RT\} \times 10^{-4}$$

where:

Δp = difference in pressures (hPa)

V_g = the volume of the gas space of the 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} = volume of added HCl (ml)

V_{KOH} = 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 the subtraction from $n_{CO2gCH4}$ the number of moles of produced methane:

$$n_{CH4} = (n_{CO_2gCH_4} + n_{CO_2l}) - n_{CO_2lCO_2g}$$

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

$$n_{CO_2gCH_4} + n_{CO_2l} = n_{total}$$

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 the 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.

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 quantity of the added substrate:

$$Y_{CH4\,g} = \frac{(V_{CH_4C} - V_{CH_4e})}{S} = \frac{V_{CH_4S}}{S} \qquad (l/g)$$

where:

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

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

Anaerobic degradability is given by the equation:

$$D_c = \frac{C_g}{C_s} \times 100$$

where:

C_s = total C content in the sample
C_g = C content in methane released during the measurement of anaerobic degradability.

The value of C_g is computed from the substrate production of methane V_{CH_4S}:

$$C_g = \frac{12\, p\, V_{CH_4S}}{RT}$$

(because 1 mol CH_4 contains 12 g C)

where:

T = temperature (°K)
R = gas constant
p = pressure
V_{CH4S} = volume of produced methane after the subtraction of endogenous production by the inoculum from total production

RESULTS AND DISCUSSION

The results of the calculation of the mass balance of elements in the digestive tract of ruminants according to the method commonly used in industrial biotechnologies (Treybal, 1968; Coulson and Richardson, 1977; Benitez, 2002) for the calculation of an anaerobic bioreactor demonstrated that at absolutely steady state the complex of bioreactions in this system can be monitored in a reliable manner only by determination of changes in the quality and quantity of organic carbon forms in dry matter of feeds and faeces in a definite balance period. However, it is to note that our preliminary experiments aimed at the investigation of nutrient and energy utilization in pigs (Nitrayová et al., 2009) and at demonstration of the influence of physiologically active substances in broiler chickens (Dlouhá et al., 2008; Ševčíková et al., 2008) were not successful.

Promising results attained in ruminants by the study of anaerobic degradability of organic matter in the input and output (feeds and faeces) and their quantity as a picture of carbon quality and quantity at steady state initiated our efforts to substantially enlarge the sets of the studied experimental variants because the Oxi Top Control Merck system with its OC 110 controller and the ACHAT OC programme make it possible to study up to 360 variants simultaneously. But it is excluded by the extremely high price of this equipment for the time being because the cost of experimental containers with measuring heads with infrared transmitters amounts to 3.6 million Czech crowns in the Czech Republic. This is the reason why we wanted to answer a question whether the anaerobic degradability of organic matter could be replaced for this purpose by determination of the ratio of the labile to stable fraction of organic matter (Kolář et al., 2009a). It would be very advantageous for operational conditions because oxidation and hydrolytic methods (Blair et al., 1995; Chan et al., 2001; Rovira and Vallejo, 2002; Shirato and Yokozawa, 2006) are much cheaper.

Different fractions of organic matter lability are examined by each of the above-mentioned methods and therefore the ratio of the labile to stable fraction is obviously different in all these methods. Unfortunately, not a single value can replace anaerobic degradabilities if we study the statically final results showing the quantity of labile and stable fractions. The situation is quite different if instead of the total quantity of the stable and labile fractions we examine the kinetics of their formation and express it by the rate constant of the labile fraction loss. For this purpose it is possible to in-

Table 1. Fodder-cropping and botanical characteristics of foothill pasture herbages for the sets A, B, C of animals of Red Pied cattle

Pasture locality	Altitude a.s.l. (m)	Pasture type	Dominant species
A	860	Arrhenatherion	*Arrhenatherum elatius, Hypericum maculatum, Achillea mitlefolium, Vicia cracca, Galium pumilum, Agrostis tenuis*
B	820	Filipendulion ulmariae	*Filipendula ulmaria, Scirpus sylvaticus*
C	770	Polygono-Trisetion	*Festuca rubra, Trisetum flavescens, Lanquisorba officinalis, Geranium sylvaticum, Alopecurus pratensis, Avena pubescens, Knautia arvensis, Polygonum bistorta*

vestigate the time changes in concentration during the oxidation of labile fractions as described by Blair et al. (1995) or time changes during biochemical oxidation that we proposed for the evaluation of organic matter degradability in previous years (Kolář et al., 2006, 2008). The results of measuring the kinetics of oxidation degradation of stable and labile fractions and their ratio could partly replace the determination of anaerobic degradability at least in preliminary experiments and to make the operation of this research technology cheaper as we will prove in the nearest future.

In the present paper the term absolutely steady state is used. It is often problematic in non-living industrial or laboratory equipment, the more so in living animals and particularly in ruminants. Therefore it is necessary to pay great attention and time to steady state, and besides the steady state of feeding all other parameters including the body condition of animals (Bouška et al., 2008; Jílek et al., 2008), genetic and nongenetic factors (Fiedlerová et al., 2008) and others should be compared.

The chemical degradability of organic matter of pasture herbage phytomass is comparable when measured by a hydrolysis-based method and by oxidation-based methods even though the measured values are naturally different (Table 2). A comparison with Table 1 shows that herbage from pasture locality C has the highest quantity of labile, easily degradable materials, which is logical in relation to the botanical composition of herbage. Table 3 documents the results of degradability of organic matter of herbage phytomass obtained only by the hydrolytic method. Obviously, the phytomass of pasture locality D is the most labile while herbage from pasture locality H is the least labile of the whole set A ... H.

Table 2. Degradability of the phytomass of foothill pasture herbages in the experimental sets of animals A, B, C according to Shirato and Yokozawa (2006) during hydrolysis and according to Blair et al. (1995) and Chan et al. (2001) during oxidation. (Reliability interval of the mean for $n = 6$ was calculated for a significance level $\alpha = 0.05$)

Method	Locality		
	A	B	C
Hydrolytic (Shirato and Yokozawa, 2006) (% labile C)	28 ± 5	23 ± 6	31 ± 6
Oxidative (Chan and al., 2001) (% labile C)	79 ± 10	80 ± 9	88 ± 9
Oxidative (Blair at al., 1995) (POC mg/g/h)	47 ± 5	40 ± 4	58 ± 5

in Tables 2 and 4 the proportion of carbon (%) of the 1st fraction LP I in total organic carbon TOC of the sample dry matter is considered as chemical degradability according to Rovira and Vallejo (2002) in the Shirato and Yokozawa (2006) modification; according to Chan et al. (2001) the percentage proportion of the sum of carbon (%) of the 1st and 2nd fraction in total organic carbon TOC of the sample dry matter is considered as phytomass chemical degradability; in the Blair et al. (1995) method the value of POC (permanganate-oxidizable carbon) mg/g/per 1 hour of the reaction is the rate of degradability by oxidation with neutral 33 nM solution of $KMNO_4$

Table 3. Distribution of organic substances of pasture herbage phytomass according to the degree of lability (Shirato and Yokozawa, 2006) to very labile (LP 1), labile (LP 2) and resistant (RP) groups in (%) in localities for the sets of animals D, E, F, G, H. (Reliability interval of the mean for n = 6 and α = 0.05)

Locality	Fraction of lability (Shirato and Yokozawa, 2006)		
	LP I	LP II	RP
D	41 ± 5	32 ± 4	27 ± 2
E	33 ± 5	36 ± 4	31 ± 4
F	25 ± 4	40 ± 4	35 ± 3
G	25 ± 4	38 ± 3	37 ± 3
H	20 ± 3	42 ± 4	38 ± 4

Table 4 illustrates the values of anaerobic degradability D_c of the samples from localities A ... H. It is demonstrated that in general the results of anaerobic degradability D_c – P (pasture herbages) are substantially higher than the values of chemical degradability, which confirms our idea that besides labile hydrolysable and oxidizable organic materials anaerobically degradable organic materials of organic matter contain a proportion of another more resistant fraction, i.e. a more stable fraction. It is interesting that the order of the samples according to chemical degradability is also maintained (with one exception) when the order according to anaerobic degradability is used:

DECAFBGH

DECAF = GBH

Table 4 shows the chemical degradability of organic matter of faeces of the animals from sets A–H. Unlike feed phytomass, the content of labile organic fraction was found to be very low if hydrolytic and oxidative methods are applied, and to fluctuate in a very narrow interval. In general, it is to state that the faeces contain a very low, and approximately identical, quantity of labile organic substances regardless of the conditions of organic matter transformation in the digestive tract of animals and regardless of feed quality.

Table 4. Anaerobic degradability of pasture herbage phytomass D_c – P, anaerobic degradability of faeces D_c – F, their difference in (%) in sets A, B, C, D, E, F, G, H. Chemical degradability of faeces in sets A to H in a hydrolytic method (Rovira and Vallejo, 2002 modified by Shirato and Yokozawa, 2006) and in both oxidative methods (Blair et al., 1995; Chan et al., 2001); (Reliability interval of the mean for n = 6 and α = 0.05)

Set	D_c – P (%)	D_c – V (%)	Difference (D_c – P) – (D_c – V)	Chemical degradability of faeces Shirato and Yokozawa (2006)		
				Rovira and Vallejo (2002) (%)	Chan and al. (2001) (%)	Blair et al. (1995) (POC)
A	53 ± 7.2	36 ± 5.1	17	3 ± 0.5	12 ± 1.3	6 ± 0.7
B	41 ± 6.8	17 ± 2.5	24	3 ± 0.4	15 ± 1.4	4 ± 0.6
C	58 ± 7.7	30 ± 4.1	28	4 ± 0.4	15 ± 1.2	5 ± 0.7
D	67 ± 9.2	54 ± 6.3	13	5 ± 0.4	18 ± 1.5	5 ± 0.4
E	62 ± 8.3	31 ± 5.0	31	4 ± 0.5	19 ± 1.4	6 ± 0.6
F	45 ± 6.5	30 ± 4.8	15	4 ± 0.4	17 ± 1.5	5 ± 0.6
G	40 ± 6.4	28 ± 4.0	12	2 ± 0.3	10 ± 1.1	3 ± 0.5
H	34 ± 5.1	24 ± 3.9	10	3 ± 0.4	16 ± 1.2	5 ± 0.5

note below Table 2 is also applicable here

Unlike the chemical degradability of faeces the anaerobic degradability of faeces D_c – F in the sets A .... H shows substantially higher values and these values are relatively different (Table 4). It is documented by the determined differences $D_cP - D_cF$. Anaerobic degradability of faeces D_cF is always lower than anaerobic degradability of feeds, and therefore we are convinced that this finding could further be utilized in research. In the set A .... H the largest difference in $D_cP - D_cF$ was observed in set E, and the lowest in set H. As mentioned above, the phytomass of herbage H was the most stable when anaerobic and chemical degradability was investigated. Now let us pay attention to the largest difference in $D_cP - D_cF$. It was determined in set A while the best degradability of feed organic matter, identically confirmed by both anaerobic and chemical degradability, was found in set D. It implies that the cause of the low difference in $D_cP - D_cF$ in set D is not the low lability of feed organic substances but another, for the time being unknown, cause of the worse utilization of feed by the animals of set D.

We can draw these conclusions from the results of this study:

(1) The comparison of the anaerobic degradability of organic matter of feeds and faeces in ruminants with the degradability of these materials determined by a hydrolytic method or oxidative methods shows that the former degradability is applicable to research observations of processes connected with the activity of the digestive tract of animals because it can be influenced by these processes, has a sufficient range of measured values, is reproducible in a satisfactory way, but the necessary instrumentation for this method is costly for the time being although the method is easy and simple. Another positive feature is that it does not bother the experimental animals at all. However, this method should further be improved and tested in exactly defined conditions.

(2) The anaerobically degradable fraction of faeces and feeds cannot be identified with the fraction of labile organic substances, which is only one of the components of the group of anaerobically degradable fractions. A marked difference is that labile fractions, determined hydrolytically or oxidimetrically, are in faeces at a minimum and practically almost identical quantity independent of feed quantity and quality or of the other living conditions of animals. On the other hand, the interval of the values of anaerobically degradable fraction in faeces is much wider and, in general, faeces differ in the quantity of anaerobically degradable fraction significantly.

(3) The quality of analytical results of the anaerobic degradability determination seems to be highly dependent on the inoculum quality. However, our experimental experiences show that it is not the case, probably because the substrate production of methane V_{CH4S} and carbon content in the gaseous phase of the methanogenic activity test (MAT) are calculated with the simultaneous subtraction of endogenous production of methane by the inoculum (Kolář et al., 2009b). It is to note that the cultivation temperature in MAT is a highly significant factor of analytical data quality. If the results are to be comparable, it is necessary to consider this method as customary and to strictly observe the working conditions defined for Oxi Top Control as described by Süssmuth et al. (1999).

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