

Anaerobic degradability and acid hydrolysis of organic manure as an illustration of dietary organic matter utilization in cows in relation to lactation period

Radka Váchalová¹, Ladislav Kolář¹, Jan Frelich¹, Jan Váchal¹, Kateřina Volfová¹, Milan Kobes¹, Václav Kudrna², Petr Homolka², Petr Marada³, Miroslav Dumbrovský⁴

¹University of South Bohemia in České Budějovice, Agricultural Faculty, České Budějovice, Czech Republic

²Research Institute of Animal Production in Prague-Uhřetěves, Praha Uhřetěves, Czech Republic

³Mendel University in Brno, Faculty of Agronomy, Department of Agriculture, Food and Environmental Engineering, Brno, Czech Republic

⁴Brno University of Technology, Faculty of Civil Engineering, Institute of Landscape Water Management, Brno, Czech Republic

Received March 18, 2014

Accepted July 23, 2014

Abstract

The objective of the present paper was to verify whether the method of studying the anaerobic degradability of organic matter of excrements could reveal the utilization of dietary organic matter in the digestive tract of ruminants in relation to the lactation period, and whether it could be replaced by a cheaper and easier method of acid hydrolysis of excrements. Changes in anaerobic degradability and hydrolysis yields of excrements were investigated in an experimental herd of 30 cows divided into two groups differing in their lactation period by 30 days. The results document that anaerobic degradability is a suitable indicator of anaerobic digestion conditions in the digestive tract of animals and of the fitness of their organism. However, the results of excrement hydrolysis according to the Rovira and Vallejo (2002) method were hardly reproducible and so they could not be used, when only the very labile organic fraction is evaluated. The evaluation of the sum of very labile fractions and semi-labile fraction provides easily available values, but these are only orientative values that cannot replace the costly and labour-consuming determination of anaerobic degradability of excrements.

Lactation, excrements, organic matter, digestive tract

Organic matter degradability was investigated mainly from the aspect of resistance during acid hydrolysis (Rovira and Vallejo 2002; Shirato and Yokozawa 2006) or oxidation (Blair et al. 1995; Chan et al. 2001).

The results of studies aimed at anaerobic degradability (Süssmuth et al. 1999) of organic matter of excrements as an illustration of their quality in relation to the condition of the digestive tract of ruminants at a steady state (Kolář et al. 2010) allow a substantial enlargement of the sets of studied experimental variants because the Oxi Top Control (Merck device) with its OC 100 controller and the ACHAT OC programme make it possible to study 360 variants at the same time. However, it is hindered by the extremely high cost of this equipment for the time being because in the Czech Republic only the experimental containers with measuring heads with infrared interface amount to 3.6 million Czech Crowns. For this reason we wanted to answer the question whether anaerobic degradability of organic matter could be replaced for this purpose by determination of the ratio of organic matter labile to stable fractions. It would be very advantageous for practical conditions because the above-mentioned oxidation and hydrolytic methods are much cheaper.

The process of anaerobic digestion of organic matter takes place in several phases with the participation of various groups of microorganisms in anaerobic environment (Baresi et al. 1978) in many forms spontaneously in nature (Garcia et al. 2000); the four fully linked phases are hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Gujer

Address for correspondence:

Prof. Ing. Ladislav Kolář, DrSc.
Agricultural Faculty
University of South Bohemia in České Budějovice
Studentská 13, 370 05 České Budějovice, Czech Republic

Phone +420 387 984 020
E-mail: lad.kolar@gmail.com
<http://actavet.vfu.cz/>

and Zehnder 1983). The hydrolytic phase consists in organic matter depolymerisation by hydrolytic enzymes (Kaseng et al. 1992); acidogenesis consists in transformation of hydrolysis products to lower organic acids and CO_2 and H_2 and lower alcohols (Kalyuzhnyi et al. 2000); acetogenesis is characterized by transformation of higher fatty acids to anions of acetic acid, formic acid, CO_2 and H_2 (Salminen et al. 2000); methanogenesis is the production of methane from anions of CH_3COO^- , HCOO^- , H_2 , CO_2 , methanol, and methylamines (Hwang et al. 2001). The sequence of reactions during digestion of ruminants is identical (Gijzen 1998).

Table 1. Breeding cows included in group A.

Cow No.	Breed	Birth date	Lactation No.	Weight at calving	Efficiency per lactation (kg)	Group
71	Holstein	28/7/2007	1	576	9 715	A
155	Holstein	22/6/2007	1	638	10 143	A
163	Holstein	29/6/2007	1	552	9 899	A
182	Holstein	28/5/2007	1	571	8 853	A
148	Holstein	12/9/2006	2	571	11 174	A
254	Holstein	18/5/2006	2	555	7 637	A
265	Holstein	19/8/2006	2	520	9 797	A
82	Holstein	3/8/2005	3	655	10 014	A
134	Holstein	5/8/2005	3	646	4 742	A
159	Holstein	16/7/2005	3	667	9 395	A
169	Holstein	5/6/2005	3	643	6 559	A
237	Holstein	17/3/2006	3	598	9 648	A
1	Holstein	27/5/2004	4	696	9 714	A
59	Czech Pied	19/8/2007	1	527	7 455	A
115	Czech Pied	26/4/2005	3	695	6 793	A
261	Czech Pied	24/9/2006	2	652	8 024	A

Table 2. Breeding cows included in group B.

Cow No.	Breed	Birth date	Lactation No.	Weight at calving	Efficiency per lactation (kg)	Group
44	Holstein	2/9/2007	1	587	13 475	B
138	Holstein	5/8/2007	1	595	7 800	B
167	Holstein	6/3/2007	1	555	8 042	B
208	Holstein	5/9/2007	1	507	8 553	B
63	Holstein	18/6/2006	2	617	11 892	B
78	Holstein	18/10/2006	2	526	8 568	B
135	Holstein	16/7/2006	2	697		B
177	Holstein	19/8/2006	2	662	8 093	B
230	Holstein	6/7/2006	2	618	10 102	B
13	Holstein	6/2/2005	3	606	10 993	B
164	Holstein	24/12/2004	3	592	9 662	B
43	Czech Pied	26/11/2005	3	676	9 167	B
51	Czech Pied	16/12/2003	4	785	7 820	B
188	Czech Pied	12/7/2006	2	716	3 670	B

Materials and Methods

The experiment was conducted in the experimental barn of the Research Institute of Animal Production in Prague-Uhřetěves. The experimental herd consisted of 30 cows, divided into two groups. The time of three weeks from the start of the experiment served only to reach a steady state. The cows were divided into two groups, group A (period 1) and control group B (period 2). Period 1 and 2 differed in the 30-day lactation period (Tables 1 and 2).

Excrement samples were taken directly from the rectum of experimental animals in order to ensure correct identification of samples and minimum contamination of samples from the ambient environment. The collected samples were fast-desiccated at reduced pressure at a temperature of 60 °C and then dried at the same temperature for 48 h. Before drying the samples were preserved at a temperature of -20 °C. Feed samples were treated in the same way.

Acid hydrolysis of organic matter and excrements was performed according to Shirato and Yokozawa (2006), who adapted the original method of Rovira and Vallejo (2002) designed for the evaluation of hydrolyzability of soil organic matter and any plant material. The method consists in hydrolysis of samples in a pyrex tube with 24.58% H₂SO₄ at a temperature of 105 °C for 30 min (easily hydrolyzable fraction LPI is hydrolyzed); in the same tube hydrolysis with 75.92% H₂SO₄ at a temperature of 20 °C, and with 10.1% H₂SO₄ at a temperature of 105 °C takes place for 1 h (semi-hydrolyzable fraction LPII is hydrolyzed). Organic matters in the rinsed residue are a hardly hydrolyzable fraction LPIII. The result is evaluated by the percentage carbon proportion of particular fractions in the total carbon content of the sample. It is to note that this is the habitual method, and any modifications of the method, e.g. replacing the difficult work in a pyrex tube by partial hydrolyses in larger vessels, provide completely incorrect results.

Anaerobic degradability is expressed by this relation:

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

C = total content of C in the sample

C_g = C content in methane released during the measurement of anaerobic degradability (Straka et al. 2009)

The analytical method of determining anaerobic degradability of excrements was evaluated by determination of the interval of reliability of the mean. It is advantageous to use for several-element sets the method according to Dean and Dixon (Eckschlager et al. 1980) based on the range (R) of results. An acid hydrolytic procedure according to Shirato and Yokozawa (2006) was performed in parallel in these tests.

Measurements of soil organic carbon

The determination of total C_{ox TOT} in soil samples was performed in agreement with ISO/DIS 14 235 by spectrophotometry after oxidation with 0.27 M K₂Cr₂O₇ in concentrated H₂SO₄ (d = 1.84 g/cm³) at 135 °C for 30 min.

Fractions of soil carbon were determined in agreement with commonly used methods: C-substances (C_{hvs}) dissolving in hot water according to the Körschens et al. (1990), fractions of C-substances based on the degree of their stability during acid hydrolysis according to Shirato and Yokozawa (2006) and during oxidation with modified Walkley-Black method according to Walkley (1947).

Humic acids (HA) and fulvic acids (FA) were isolated after extraction of soil samples with a mixture solution of 0.1M Na₄P₂O₇ · 10H₂O + NaOH by precipitation with H₂SO₄ and separation of HA:FA using the classical method by Kononová and Bělčíková (1963). Carbon in HA and FA was again determined using the method according to ISO/DIS 14 235.

Results and Discussion

The results in Table 3 document the high reproducibility of acid hydrolysis of excrements, a small range of the variation of results and a small standard deviation. As an analytical method, it is obviously preferable to anaerobic degradability (D_c), the results of which have the range and standard deviation higher more than three times. But results in Table 3 show that the sensitivity of acid hydrolysis is very low. This method did not register at all a change in the lactation period by 30 days and associated changes in organic matter utilization in the digestive tract of an animal.

Table 4 shows that anaerobic degradability substantially decreases with a 30-day difference in the lactation period; In conclusion we can say, that the regeneration of the digestive tract of the animals made progress and therefore the activity of the digestive tract as an anaerobic reactor was improved.

Table 3. Testing of analytical methods – determination of anaerobic degradability and acid hydrolysis in excrements of animal No. 82 from single sampling, in period 1 and in period 2 longer by 30 days of lactation.

	Period	Sample								R	S _R	L _{1,2}
		1	2	3	4	5	6	7	8			
Dc	1	32.3	38.4	35.4	36.7	39.1	38.2	37.7	34.9	6.8	2.38	36.6 ± 2.0
(% C _{tot})	2	26.2	31.5	28.4	29.4	32.3	30.7	33.2	35.1	8.9	3.12	30.8 ± 2.6
LPI	1	4.1	3.9	3.8	4.3	4	3.9	4.1	4.2	0.4	0.14	4.0 ± 0.1
(% C _{tot})	2	4.2	4	4.1	3.9	4	3.7	3.9	3.8	0.5	0.18	4.0 ± 0.1
LPII	1	34.5	34.1	34.6	35.4	33.8	34	35.1	34.9	1.6	0.56	34.5 ± 0.5
(% C _{tot})	2	32.1	34	33.2	33.7	32.8	33.9	32.7	32.5	1.9	0.67	33.1 ± 0.6
LPIII	1	61.4	62	61.6	60.3	62.2	62.1	60.8	60.9	1.8	0.63	61.4 ± 0.5
(% C _{tot})	2	63.7	62	62.7	62.4	63.2	62.4	63.4	63.7	1.7	0.6	62.9 ± 0.5

D_c – anaerobic degradability; R – range, L_{1,2} – interval of reliability of the mean at $P = 0.05$ ($L_{1,2} = \bar{x} \pm K_n \cdot R$; K_n ($n = 8$) = 0.29 ($P = 0.05$)); S_R – standard deviation from the range ($S_R = k_n \cdot R$; k_n ($n = 8$) = 0.351); LPI – labile fraction; LPII – semi-labile fraction; LPIII – stable fraction

Table 4. Anaerobic degradability and acid hydrolysis of excrements in period 1 and in period 2 longer by 30 days of lactation in five successive samples in three experimental animals at a steady state.

	Animal No.	Period	Sampling					R	S _R	L _{1,2}
			1	2	3	4	5			
Dc (% C _{tot})	82	1	35.2	36	38.3	34.9	32.5	5.8	2.49	35.4 ± 3.0
		2	29.4	30.7	26.2	29.9	30.4	4.5	1.93	29.3 ± 2.3
	134	1	41.7	38.2	40	36.4	32.9	8.8	3.78	37.8 ± 4.5
		2	33.3	30.3	29.4	28.1	31.5	5.2	2.24	30.5 ± 2.7
	237	1	28.4	27.2	32.6	27.1	30	5.5	2.36	29.1 ± 2.8
		2	24.7	21.5	20.1	22.3	23.9	4.6	1.98	22.5 ± 2.3
LPI (% C _{tot})	82	1	3.8	3.9	3.9	3.8	3.8	0.1	0.04	3.8 ± 0.0
		2	3.7	3.9	3.8	3.8	3.9	0.2	0.09	3.8 ± 0.1
	134	1	3.7	3.8	3.7	3.9	3.8	0.2	0.09	3.8 ± 0.1
		2	3.6	3.8	3.6	3.8	3.7	0.02	0.09	3.7 ± 0.1
	237	1	3.8	3.7	3.7	3.6	3.7	0.2	0.09	3.7 ± 0.1
		2	3.8	3.8	3.7	3.7	3.8	0.1	0.04	3.8 ± 0.0
LPII (% C _{tot})	82	1	34.2	35.2	33.8	34.9	35.4	1.6	0.69	34.7 ± 0.8
		2	33.3	33.9	34.8	35.1	36.2	2.9	1.25	34.7 ± 1.5
	134	1	37.6	38.2	35.2	36.4	38.1	3	1.29	37.1 ± 1.5
		2	38.5	38.8	39.9	36.8	37.4	3.1	1.33	38.3 ± 1.6
	237	1	32.7	31.8	33.1	30.8	32.5	2.3	0.99	32.2 ± 1.2
		2	31.7	33.3	34.9	35.1	32.9	3.4	1.46	33.6 ± 1.7
LPIII (% C _{tot})	82	1	62	60.9	62.3	61.3	60.8	1.5	0.64	61.5 ± 0.8
		2	63	62.2	61.4	61.1	59.9	3.1	1.33	61.5 ± 1.6
	134	1	58.7	58	61.1	59.7	58.1	3.1	1.33	59.1 ± 1.6
		2	58.9	57.4	56.5	59.4	58.9	2.9	1.25	58.2 ± 1.5
	237	1	63.5	74.5	63.2	65.6	63.8	2.4	1.03	64.1 ± 1.2
		2	64.5	62.9	61.4	61.2	63.3	3.3	1.42	62.7 ± 1.7

D_c – anaerobic degradability; R – range, L_{1,2} – interval of reliability of the mean at $P = 0.05$; ($L_{1,2} = \bar{x} \pm K_n \cdot R$; K_n ($n = 5$) = 0.430 ($P = 0.05$)); S_R – standard deviation from the range ($S_R = k_n \cdot R$; k_n ($n = 8$) = 0.351); LPI – labile fraction; LPII – semi-labile fraction; LPIII – stable fraction

Table 5. The influence of inoculum quality on determination of anaerobic demonstrated by the use of three different inocula for determination of anaerobic degradability of excrements in three experimental animals at a steady state in period 2 of group A.

	Inoculum	Animal No.	R	S _R	L _{1,2}	Order of anaerobic degradability
Dc (% C _{tot})	A	82	4.5	1.93	29.3-2.3	134>82>237
		134	5.2	2.24	30.5-2.7	
		237	4.6	1.98	22.5-2.3	
	B	82	2.9	1.25	25.1-1.5	134>82>237
		134	4.1	1.76	32.6-2.1	
		237	4.8	2.06	16.4-2.4	
	C	82	6.2	2.66	24.1-3.2	134>82>237
		134	7.4	3.18	26.3-3.8	
		237	5.5	2.36	28.9-2.8	

D_c – anaerobic degradability; R – range, L_{1,2} – interval of reliability of the mean at $P = 0.05$; S_R – standard deviation from the range

Data in Table 5 document how important the inoculum quality is. When different inocula are used, not only the rate of anaerobic degradability but also the obtained values for various sources of the tested sample are different. It is, however, crucial that the same order of anaerobic degradability is always maintained in all used inocula.

The results of acid hydrolysis in Table 6 show that the results of labile fraction LPI determination had the widest range; in contrast, the results of stable fraction LPIII had a much smaller range. It confirms our opinion that we cannot assess solely fraction LPI as a crucial criterion as Kolář et al. (2010) did. Much better information is provided by the sum of LPI and LPII fractions, even though in comparison with the results of anaerobic digestion it is only an orientative result.

Table 6. Anaerobic degradability and acid hydrolysis of the administered feed during 45 days of experiment.

Feed		Sample collection				R	S _R	L _{1,2}
		3.11.2010	6.11.2010	14.11.2010	18.11.2010			
Dc (% C _{tot})	A	55.8	48.3	60.4	56.7	12.1	5.8	55 ± 311.1
	B	55.3	45.2	61.4	53.2	16.2	7.8	53.8 ± 14.9
LPI (% C _{tot})	A	35.4	47.6	39.8	38.2	12.2	5.8	40.2 ± 11.2
	B	34.9	41.7	40.1	41.1	6.8	3.3	39.4 ± 6.3
LPII (% C _{tot})	A	38.4	32.9	30.8	34.7	7.6	3.6	34.2 ± 7
	B	37.8	33.2	31.9	32.3	5.9	2.8	33.8 ± 5.4
LPIII (% C _{tot})	A	26.2	23.5	29.4	27.1	5.9	2.8	26.5 ± 5.4
	B	27.3	24.1	28	26.6	3.9	1.9	26.5 ± 3.6

D_c – anaerobic degradability; A – 1 + 2 sampling; B – 3 + 4 sampling; R – range, L_{1,2} – interval of reliability of the mean at $P = 0.05$; (L_{1,2} = $\bar{x} \pm K_n \cdot R$; K_n (n = 4) = 0.48 ($P = 0.05$)); S_R – standard deviation from the range ($S_R = k_n \cdot R$; k_n (n = 8) = 0.92); LPI – labile fraction; LPII – semi-labile fraction; LPIII – stable fraction

Comparing Tables 7 and 8, it is evident that the time span of 30 days of lactation did not influence labile fraction LPI of acid hydrolysis at all but the sum of fractions LPI and LPII indicates that stable fraction LPIII was slightly higher in period 2, i.e. the longer lactation period was reflected in better utilization of labile organic matters in the digestive tract of animals. The range of results in Tables 7 and 8 is small because statistical outliers were excluded. However, there was a difference of about 5%. In anaerobic digestion the lactation

duration resulted in about 7% better utilization of feed. These are small differences and this is the reason why Table 9 contains the consistency test of results of anaerobic digestion in period 1 (Table 7) and in period 2 (Table 8). Lord's test (Eckschlager et al. 1980) was used for testing:

$$\mu = \frac{|\bar{x}_A - \bar{x}_B|}{R_A + R_B}$$

and critical values of μ , for $P = 0.05$ and for $n = 9$. Because the determined values $\mu = 0.983$ and $\mu = 0.404$ are higher than the critical value $\mu = 0.167$, it is possible to state

Table 7. Anaerobic degradability and acid hydrolysis of excrements in initial period 1 with feed A of the first half attempt and with feed B of the second half attempt.

	Feed	n	R	k_n	S_R	$t_{\alpha(n-1)}/\sqrt{n}$	$L_{1,2}$
Dc (% C_{tot})	A	9	3.3	0.34	1.12	0.77	32.0 ± 0.9
	B	8	6.8	0.35	2.38	0.84	31.0 ± 2.0
LPI (% C_{tot})	A	9	0.2	0.34	0.07	0.77	3.8 ± 0.0
	B	10	0.3	0.32	0.1	0.72	3.8 ± 0.0
LPII (% C_{tot})	A	8	2	0.35	0.7	0.84	33.2 ± 0.6
	B	8	2.1	0.35	0.73	0.84	35.1 ± 0.6
LPIII (% C_{tot})	A	10	2.4	0.32	0.77	0.72	63.0 ± 0.6
	B	10	2.2	0.32	0.7	0.72	61.1 ± 0.5

D_c – anaerobic degradability; A – 1 + 2 sampling; B – 3 + 4 sampling; R – range; $L_{1,2}$ – interval of reliability of the mean at $P = 0.05$; S_R – standard deviation from the range; LPI – labile fraction; LPII – semi-labile fraction; LPIII – stable fraction; n – number of samples after the exclusion of outliers; k_n – coefficient for the calculation; S_R – standard deviation from the range; $t_{\alpha(n-1)}/\sqrt{n}$ – coefficient from Student's t -distribution for $P = 0.05$

Table 8. Anaerobic degradability and acid hydrolysis of excrements in period 2 longer by 30 days of lactation compared to period 1 with feed A and with feed B.

	Feed	n	R	k_n	S_R	$t_{\alpha(n-1)}/\sqrt{n}$	$L_{1,2}$
Dc (% C_{tot})	A	9	2.8	0.34	0.95	0.77	26.1 ± 0.7
	B	9	3.1	0.34	1.05	0.77	26.6 ± 0.8
LPI (% C_{tot})	A	10	0.2	0.32	0.06	0.72	3.7 ± 0.0
	B	10	0.2	0.32	0.06	0.72	3.8 ± 0.0
LPII (% C_{tot})	A	8	1.9	0.35	0.66	0.84	29.1 ± 0.6
	B	9	2.2	0.34	0.75	0.77	28.3 ± 0.6
LPIII (% C_{tot})	A	10	2.8	0.32	0.9	0.72	67.2 ± 0.6
	B	9	2.5	0.34	0.85	0.77	67.9 ± 0.7

D_c – anaerobic degradability; A – 1 + 2 sampling; B – 3 + 4 sampling; R – range; $L_{1,2}$ – interval of reliability of the mean at $P = 0.05$; S_R – standard deviation from the range; LPI – labile fraction; LPII – semi-labile fraction; LPIII – stable fraction; n – number of samples after the exclusion of outliers; k_n – coefficient for the calculation; S_R – standard deviation from the range; $t_{\alpha(n-1)}/\sqrt{n}$ – coefficient from Student's t -distribution for $P = 0.05$

that the revealed apparently small difference in results is significant at $P = 0.05$ and the null hypothesis can be rejected. The longer lactation period had a significant influence on better utilization of organic matter in the digestive tract of experimental animals.

We were convinced that the lower appetite of animals to feed intake in the puerperal period would be reflected in the self-regulation of the digestive tract so that the higher biochemical activity of the organism would ensure better utilization of feed ration. This hypothesis was not confirmed; on the other hand, with time elapsing from parturition the organism regenerates and the enzymatic activity of the digestive tract of experimental animals is gradually increasing.

The calculation of the consistency tests of results in period 1 and 2 could show that the results of differences in stable fraction LPIII are significant at $P = 0.05$; so it would theoretically be possible that difficult determination of anaerobic digestion could be replaced by an easier method of acid hydrolysis. But sensitivity of the method is problematic because in other

Table 9. Evaluation of the influence of lactation duration on a change in the results of anaerobic degradability of excrements by testing the consistency of results in period 1 and period 2 differing in 30 days of lactation, feed A and feed B.

X_A	X_B	R_A	R_B	μ	μ_{crit}
32	26	3.3	2.8	0.983	0.167
31	27	6.8	3.1	0.404	

μ_{crit} – critical values of μ (μ_{crit} for $n = 9$ and $P = 0.05$)

experiments we obtained non-significant results of the used acid hydrolysis of excrements. Acid hydrolysis of the excrement can replace expensive determination of anaerobic degradability of the excrement.

Acknowledgement

The study was supported by Grant of Ministry of Agriculture of the Czech Republic (Project No. QJ1230066). We are very grateful for this support.

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