Suitability of eight techniques for estimating digestibility of herbage from continuously grazed all-day pasture

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ABSTRACT

The objective of this study was to estimate digestibility of herbage using eight different methods. Organic matter digestibility (OMD) was estimated with titanium (Ti) dioxide and acid-insoluble ash (AIA) as indigestible markers, four faecal nitrogen (N) equations, which use the same raw data, the pepsin-cellulase method (*in vitro* OMD) and digestibility trials with wethers (*in vivo* OMD). An all-day pasture with continuous stocking at 2.8 cows/ha was chosen for the comparison because it restricted selection during grazing and thus allowed comparison of *in vitro*- and stall feeding-based methods with methods used for pastures. A crossover experiment with eight lactating Simmental cows was conducted from May until July 2008, with two consecutive experimental periods of 28 days. The cows were divided in two similar experimental groups. Four cows were put into individual stalls and fed herbage clipped from the experimental pasture and feed intake was measured. The other four cows were put onto the fenced pasture. All cows were supplemented with 2 kg/d fresh matter of grain maize. After four weeks, the treatment groups were switched.

OMD differed considerably between methods (by 20–110 g/kg). Applying the same method, OMD on pasture differed from OMD in-stall indicating that the grazing animal cannot be replaced by mowing even with high experimental effort and low opportunity for selection. It also differed over time. Only the faecal N methods were readily applicable on pasture at sufficiently high temporal resolution and – with one exception – produced similar results in-stall as the Ti method. They can in general be recommended for a large range of conditions including grazing studies but the variety of existing equations makes it difficult to select the appropriate one. This calls for the development of better defined and rigorously tested equations.

1. Introduction

The increasing costs of feed concentrates have led to increased interest in pasture-based milk production, particularly with respect to cost-effective pasture management systems. Feed intake and organic matter digestibility (OMD) ingested are the two most important components affecting the animal performance, but neither can be measured easily at pasture

(Moore, 1996). Attempts to predict intake and OMD are quite numerous (Moore, 1994; Mayes and Dove, 2000), but all have their weaknesses (Moore, 1996; Delagarde and O'Donovan, 2005).

Feed intake at pasture can be modelled (Delagarde and O'Donovan, 2005) or it can be estimated from the excretion of indigestible markers, if the OMD of the ingested feed is known. Hence, knowledge of OMD is important in its own right and to estimate feed intake from the excretion of markers. The underlying concept is that when a known amount of an indigestible marker is fed and equilibrium is achieved, the same amount of that marker should then be voided with the faeces.

Several methods, each based on different principles, allow the determination of OMD. Each method is a simplification that reduces measurement effort and thus allows more repetitions, but it then introduces uncertainty regarding whether the obtained results can be generalized in a broader sense. The complexity of the examined situation increases from measuring *in vitro* OMD to *in vivo* techniques, the application of markers and the faecal N methods.

In vitro OMD methods are the least specific because they ignore the technical aspects of production and disregard the animal, except as a donor of rumen liquor. These techniques either simulate the rumen fermentation by enzymes (De Boever et al., 1986) or the rumen liquor is used to measure OMD microbiologically (Tilley and Terry, 1963; Menke et al., 1979). *In vivo* techniques, using sheep (wethers) or cattle, are more realistic since they are based on direct measurement of feed intake and faecal output in the stall under controlled conditions. These methods account for the animal digestive process, but wethers are often taken as the "standard ruminant" simply because of their ease of handling and management. The influence of species, lactation, breed, sex, age and level of nutrition are seldom considered. Furthermore, the standardized conditions for these "*in vivo*" tests may deviate considerably from real conditions that are encountered in normal animal keeping (Gabel et al., 2003).

Markers account for these influences. Internal markers, such as lignin, chromogen or acid-insoluble ash (AIA), are unavoidably taken up together with the feed while external markers, such as metal oxides or rare-earth compounds are fed to the animals. N-alkanes are widely used markers, in which a combination of an internal and an external marker is used. Major limitations of such marker studies include non-representative sampling of the ingested feed, incomplete faecal recovery of the marker and differing recovery rates of the markers (Lancaster, 1949; Titgemeyer et al., 2001; Mayes and Dove, 2000).

Recently, faecal-N methods gained interest as indirect methods (Schmidt et al., 1999; Lukas et al., 2005; Wang et al., 2009) for allowing the calculation of OMD from faecal-N content using regression models. These faecal-N methods can be applied under a wide range of conditions, including grazing, given that fresh faeces can be sampled.

Stall experiments are comparatively easy to control through feed intake measurements and faecal sampling. However, the applicability of OMD obtained from stall-feeding studies to OMD under natural grazing has not yet been established. In many cases, the provision of cut forage samples to animals may not entirely supply the same feed quality as forage ingested during grazing. The same applies to other methods that employ forage samples, such as *in vitro* or *in sacco* digestibility tests. Cut or hand-plucked herbage may not simulate selection by the grazer (Baumont et al., 2000; Schlegel et al., 2000). Oesophageally fistulated animals avoid this source of error, but they require a surgical preparation of the animal and irritation of the animals may influence the results.

Comparisons of two or three methods for OMD estimation have already been done (Macoon et al., 2003; Schiborra et al., 2010). However, Schiborra et al. (2010) compared *in vivo* and *in vitro* OMD, but attributed the observed differences entirely to the grazing selection by the sheep. They assumed that both methods would lead to identical results if selection did not occur. Furthermore, studies that include a large range of methodologies are missing, to our knowledge. Thus, the question remains whether *in vivo* and *in vitro* OMD methods, and other OMD methods, yield similar results given similar conditions

Making this type of comparison would be relatively simple using confined animals but it is significantly more challenging under grazing conditions. In the present work, we introduce a new approach that allowed us to establish the same feed for animals both in-stall and at pasture, thus allowing comparison of several techniques for OMD estimation under grazing and stall-fed conditions.

The objectives of this research were: (1) to estimate OMD of herbage from all-day pasture using different techniques and (2) to compare the results of the different techniques.

2. Materials and methods

2.1. Experimental site

The experiment was conducted at the research station of the University of Applied Sciences Weihenstephan, near Freising, southern Germany (48°26′N; 11°46′E), 493 m above sea level. The experimental pasture was a 3.0 ha semi-natural grassland on silty loam soils. The mean annual temperature and precipitation are 7.5 °C and 794 mm/yr, respectively. The pasture plot was fertilized with 54 kg/ha N (as calcium ammonium nitrate) before the experiment started and again with 54 kg/ha N after 28 days of grazing. The botanical composition of the pasture (Table 1), estimated by visual inspection by trained persons before and after the experiment, was dominated by grasses (0.82–0.86 in the harvested forage dry matter mass) with *Lolium perenne* (L.) contributing the most (0.60–0.75), while the legume *Trifolium repens* (L.) contributed 0.05–0.08.

Compressed sward height was measured daily before the evening milking with a rising-plate meter (Ashgrove, RD 10, New Zealand) applying a load of 4.8 kg/m². Approximately 150 measurements per day were taken, one at every ten steps along two diagonal lines across the experimental pasture. The target sward height was 6–7 cm.

Table 1
Botanical composition of the experimental pasture (fraction of standing dry matter).

Date	Start of experiment (28.04.2008)	End of experiment (18.07.2008)
Grasses		
Lolium perenne	0.750	0.600
Poa trivialis	0.032	0.086
Poa annua	0.016	0.019
Poa pratensis	0.000	0.036
Dactylis glomerata	0.062	0.079
Three other species	0.007	0.003
Sum grasses	0.867	0.823
Herbs		
Plantago major	0.020	0.060
Taraxacum officinale	0.003	0.017
Five other species	0.009	0.012
Sum herbs	0.032	0.089
Legumes		
Trifolium repens	0.084	0.054
Sum legumes	0.084	0.054
Miscellaneous	0.017	0.034
Total	1.000	1.000

2.2. Experimental animals

The experiment was initiated on 19 May 2008 and ended on 18 July 2008 and consisted of two continuous 28-day trial periods (experiment period 1 and 2) each consisting of one week of acclimatisation to the experimental conditions and three weeks of measurement (measuring period 1 and 2). Eight pregnant, lactating multiparous Simmental cows in their second or third lactation were combined in a total herd of 30 cows. At the start of the experiment, the cows were between 80 and 133 days in lactation. Energy corrected milk (ECM) was calculated by standardizing actual milk production of a cow to 35 g/kg milk fat and 32 g/kg milk protein following Bernard (1997). ECM averaged 28 kg/d during the seven days before the commencement of the experiment. The animals were accustomed to grazing and the feed by keeping them on a pure pasture diet for 6 weeks before the experiment started.

The cows were matched for age, body weight (BW), calving date and milk production during the current lactation and then allocated to one of the two groups of four cows. One group of four cows was put onto the fenced experimental pasture plot during period 1, while the other four cows were penned individually indoors to measure feed intake. The individual boxes in the stall had a floor area of 20 m^2 and the cows were allowed to move freely. The cows in the stall were fed with clipped herbage collected on the experimental pasture by mowing between the grazing cows. This was done to obtain, as closely as was possible, the same feed for the stall-fed and pasture groups. This strategy led to a total stocking density of 2.8 cows/ha. All cows had continuous access to fresh herbage and fresh water. After four weeks, the groups were switched, so that the four cows previously grazing on the pasture were moved to the stalls (P-S group), and the cows, that had been penned in the stall during period 1 were moved to the pasture (S-P group).

The dry matter (DM) intake in the stall was measured by weighing feed offered and feed refused after each meal before fresh herbage was fed. Feed on offer aimed to produce at least 10% feed remains. DM content was determined by drying at 60 °C for 48 h.

The cows on the pasture were allowed to graze from 07:00 to 16:00 and from 18:00 to 05:00, with two milkings in between. The pasture had water tubs fitted with float-control devices to ensure permanent availability of fresh drinking water.

All cows were milked in a 2 × 4 herringbone milking parlour. Milk production was recorded using flowmeters (Westfalia, Germany) and milk samples were taken four times a week twice a day in the morning and afternoon, for analysis of fat, protein and urea contents and pH value. Fat and protein contents were determined by infrared spectrophotometry (MilcoScan-FT-6000; Foss Electric, Hillerod, Denmark) by the Bavarian Association for Raw Milk Testing, Wolnzach, Germany, in order to convert milk production to ECM.

After each milking, the cows from both groups were confined for 30 min and half of the daily allotment of grain maize (1.72 kg/d DM) was fed in individual troughs. After this feeding, the cows were brought to their respective treatments. The grain maize served as the carrier for the Ti marker (twice daily dosing) and as an energy supplement to balance the high content of ruminally degradable protein in herbage. Weekly samples of maize were dried at 60 °C for 48 h and the DM content was calculated. Feed remains of grain maize were collected and weighed and DM content was estimated by drying to calculate the effective maize and marker intake.

During the entire experimental periods all cows were weighed twice weekly on two consecutive days and the average BW for the two days was determined. Body condition score and back fat thickness were determined at the beginning of the last week of each period by the same two independent evaluators. Body condition score on a scale of 1–5 (1, very thin; 5,

Table 2
Feed properties of herbage harvested from all-day pasture above a stubble height of 3 cm and of grain maize.

Item	Herbage									Grain maize
Week:	1	2	3	4	5	6	7	8	Mean	
Period:	0	1	1	1	0	2	2	2		
Sward height (cm)	6.2	6.4	6.9	6.3	6.4	6.2	6.1	5.7	6.2	
Dry matter content of fresh matter (g/kg)	153	165	168	164	169	161	178	152	164	887
Dry matter composition (g/kg)										
Organic matter	902	903	905	896	899	901	893	895	899	982
Crude protein	216	188	190	189	201	211	213	230	205	94
Ether extract	39	36	42	37	41	43	38	46	40	46
eIOM	242	315	339	287	249	242	233	224	266	
NDF_{om}	533	554	501	506	499	549	517	522	523	336
NFC	114	125	172	165	159	97	125	96	131	506
ADF_{om}	307	297	310	311	284	304	271	283	296	38
Lignin(sa)	26	27	26	25	26	22	23	24	25	16

eIOM, enzymatically insoluble organic matter; NDF_{om}, neutral detergent fiber not assayed with a heat stable amylase and expressed exclusive of residual ash; NFC, non fiber carbohydrates; ADF_{om}, acid detergent fiber not assayed with a heat stable amylase and expressed exclusive residual ash; Lignin(sa), Lignin determined by solubilization of cellulose with sulphuric acid. Period 0, adaptation; period 1 and 2, digestibility measuring periods.

very fat) was defined by the method of Wildman et al. (1982) and back-fat thickness was measured sonographically (Esaote, Tringa Linear 50, Oberhausen, Germany) at the measuring point defined by Schröder and Staufenbiel (2006).

2.3. Experimental feed

Herbage was clipped on the experimental pasture with a green fodder harvester (Hege 212B, Waldenburg, Germany) to a stubble height of 30 mm. Excess feed on offer was reduced at the beginning of experimental week 4 by additionally harvesting 15% of the pasture. Feed properties are shown in Table 2.

The main part of the harvested herbage (fresh matter approximately 450 kg/d) was fed *ad libitum* to the four cows in the stall to measure feed intake. Representative samples of the harvested forage were taken daily to determine DM content by oven drying. For the digestion experiment with wethers, herbage (15 kg/d) was stored in plastic bags at $-18\,^{\circ}$C.

2.4. Sample collection of herbage and faeces

A sample of the harvested herbage was immediately frozen and stored at $-18\,^{\circ}$C for later chemical analyses. The frozen samples were thawed (36 h at room temperature), dried at $60\,^{\circ}$C for 48 h and then ball milled to a homogenous fine powder. Weekly samples were pooled and the gross composition (DM, crude protein, ether extract, ash) was measured. AIA contents (Van Keulen and Young, 1977) and acid-detergent fiber (ADF_{om}), neutral detergent fiber (NDF_{om}) and lignin(sa) (Robertson and Van Soest, 1981; Van Soest et al., 1991) were measured. ADF_{om} and NDF_{om} were not assayed with a heat stable amylase and are expressed exclusive of residual ash. Lignin was determined by solubilization of cellulose with sulphuric acid. Non fiber carbohydrate (NFC) content was calculated as NFC = OM – CP – NDF_{om} – ether extract. The content of enzymatically insoluble organic matter (eIOM) was determined with the pepsin-cellulase method of De Boever et al. (1986).

About 50 g faeces per cow, obtained from all animals by rectal grab sampling in the parlour, were taken four times a week during the measuring periods, at 06:30 and 17:30, after milking. This grab sampling method avoided contamination of faeces by urine, insects or soil. All sampling was carried out by the same person in the same way and with the same tools. The samples were immediately stored in plastic containers and frozen at $-18\,^{\circ}$C until analysis.

Daily excretion of faeces was measured by feeding 9 g Ti (as TiO_2) twice daily as external marker to every cow from the start of the experiment. The amount of an indigestible marker voided with the faeces, when fed continuously over time, should be constant after flow equilibrium is achieved (Rothfuss et al., 1997; Myers et al., 2004; Glindemann et al., 2009). Thus, the concentration of the marker in faeces directly relates to faeces output (see Eq. (1)). The digestibility can be calculated when feed intake is measured simultaneously.

Faeces samples during the measuring periods were pooled by animal and by week. The samples of the acclimatization periods were not considered. This resulted in 48 pooled samples (8 cows × 3 weeks/period × 2 periods) that were analyzed for N, AIA (Van Keulen and Young, 1977) and the Ti content (absorption spectrophotometry).

Faecal DM output was calculated as follows (Lippke, 2002):

$$\text{faecal DM output (kg/d)} = \frac{[\text{Ti dosed per day (g/d)}]}{[\text{mean Ti content in faeces (g/kg)}]} \times \text{recovery rate} \qquad (1)$$

An average recovery of 960 g/kg was used in this study to calculate faecal DM output. The pasture DM intake was determined using the calculated faecal output and *in vitro* DM digestibility ($DDM_{in\ vitro}$) of pasture herbage according to the equation by Lippke (2002):

$$\text{DM intake (kg/d)} = \frac{[\text{faecal DM output (kg/d)}]}{[(1 - DDM_{in\ vitro})]} \tag{2}$$

2.5. Determination of digestibility with wethers

Eight wethers, weighing 70–80 kg, were matched for age and BW and then divided into two similar groups of four wethers for *in vivo* digestibility determination. The animals were kept in single metabolic cages. Feed was given to the wethers twice a day in equal amounts at 07:00 and 18:00. Although other studies have often used dried herbage, we used thawed herbage because Beever et al. (1976) have shown that there is no influence of freezing and thawing on OMD. The frozen herbage, harvested as described above, was thawed 24 h in closed bags before feeding.

The first group of wethers received a pure herbage diet and 15 g/d mineral supplement consisting of (g/d): Ca 2.70; P 0.77; Mg 0.29; Na 1.50 and the following trace elements (mg/d): Zn 120; Mn 25.5; Co 0.4; I 0.9 and Se 0.9, as declared by the manufacturer (Hoeveler Spezialfuttermittel GmbH und Co KG, Dormagen, Germany). The second group of wethers was fed grain maize in addition to the herbage and mineral supplement. To ensure a similar ration composition for both wethers and cows, the wethers of group 2 received grain maize to contribute, on average, a fraction of 0.124 (SD 0.006; $n = 4$) to the total diet (DM basis). Fresh water was freely available to both groups.

The nutrition level during the experiments was adjusted to provide 1.1–1.2 times maintenance, according to Gabel et al. (2003) because the OMD is largest when energy level is near the maintenance requirement. Feeding times were 07:30 and 18:30 h and faecal collections were made at 07:00 and 18:00.

The experimental period for the wethers consisted of 14-day adaptation to the diet, followed by 8 days of intake measurements. The amounts of forage offered, the feed remains, and faeces excreted by each wether were weighed daily. DM of fresh herbage was determined twice daily by drying for 48 h at 60 °C. The wethers were harnessed with faecal bags, which were tightened with three cordons at their body. The faecal bags were emptied twice daily and faeces were immediately frozen at −18 °C. A single representative sample of faeces from each animal was obtained by pooling 20% of each defecation. Subsamples were stored at −18 °C until DM determination. After 72 h freeze-drying, the faeces and the herbage samples were analyzed for AIA as described for the cows' faeces.

The whole study was carried out according to the standards of CCAC (1993).

2.6. Calculation of digestibility

Digestibility of organic matter (OMD) was estimated with Ti and AIA as indigestible markers, four faecal nitrogen (N) equations, which use the same raw data, the pepsin-cellulase method (*in vitro* OMD) and the digestibility trials with wethers (*in vivo* OMD). All equations calculate digestibility as a fraction of OM. The equation to calculate OMD with the pepsin-cellulase method (Weissbach et al., 1999) was:

$$OMD_{in\ vitro} = \frac{(940 - A - 0.62 \times eIOM - 0.000221 \times eIOM^2)}{(1000 - A)} \tag{3}$$

where eIOM is enzymatically insoluble OM in DM (g/kg) and A is ash in DM (g/kg).

There are several equations to calculate OMD from the faecal N content. The equation by Schmidt et al. (1999) applies to fresh herbage:

$$OMD_{N1} = 0.8955 - \frac{4.6}{x}, \tag{4}$$

where x is N content in faeces OM (g/kg). Subsequently this method is termed 'faecal N method for fresh herbage'.

The equation after Schmidt and Jentsch (1994) applies for conserved forage-based diets:

$$OMD_{N2} = 0.9 - \frac{5.13}{x} \tag{5}$$

Subsequently, we term this method the 'faecal N method for forage-based diets'.

Although the following two methods are also based on the N content, we term them crude protein methods because the original equations were based on crude protein, which is obtained as faecal N content × 6.25.

The equation to calculate OMD with the faecal crude protein method by Lukas et al. (2005) is:

$$OMD_{N3} = a_i - 1.077\ \exp^{(-0.01515p)} \tag{6}$$

where a_i is a location factor and p is crude protein content in faeces organic matter (g/kg). The location factor a_i is 0.7976 for Braunschweig and Hohenheim (Germany) and 0.7286 for Gumpenstein (Austria). The method is later termed OMD_{N3BH} when using a_i for Braunschweig and Hohenheim and OMD_{N3G} when using a_i for Gumpenstein. Both Braunschweig/Hohenheim

Table 3

In vivo dry matter digestibility of two diets in the wether test ($n = 4$ for each diet).

Item	Herbage			Herbage and maize			P
	Content (g/kg)	Mean digestibility	SD	Content (g/kg)	Mean digestibility	SD	
Organic matter	904	0.777	0.008	913	0.794	0.013	0.0438
Crude protein	212	0.796	0.007	198	0.788	0.011	0.1477
NFC	131	0.684	0.025	175	0.794	0.004	0.0004
Ether extract	39	0.394	0.040	39	0.536 (0.473)	0.026 (0.128)	0.1428
NDF_{om}	522	0.821	0.011	501	0.817	0.021	0.3725
ADF_{om}	273	0.769	0.011	246	0.791	0.012	0.0235
Ash	96	0.467	0.033	87	0.465	0.026	0.4618

NFC, non fiber carbohydrates; NDF_{om}, neutral detergent fiber not assayed with a heat stable amylase and expressed exclusive residual ash; ADF_{om}, acid detergent fiber not assayed with a heat stable amylase and expressed exclusive residual ash. Values in parentheses are influenced by one outlier.

and Gumpenstein share similarities with our experimental site. We found no criteria to decide beforehand which a_i was better suited in our case.

The equation to calculate *in vivo* OMD from the wether digestion test is

$$OMD_{in\ vivo} = 1 - \frac{[\text{faecal output OM (kg/d)}]}{[\text{feed intake OM (kg/d)}]} \tag{7}$$

Accordingly, as OM intake and faecal output from Eq. (1) for the cows in the stall was known, OMD_{Ti} was calculated as:

$$OMD_{Ti} = 1 - \frac{[\text{faecal output (kg/d)} - \text{faecal ash output (kg/d)}]}{[\text{dry matter intake (kg/d)} - \text{feed ash intake (kg/d)}]} \tag{8}$$

OMD_{AIA} was calculated as:

$$OMD_{AIA} = 1 - \left[\frac{\text{AIA content in feed OM (g/kg)}}{\text{AIA content in faeces OM (g/kg)}}\right] \tag{9}$$

2.7. Statistical methods

Linear regressions (weighted least-squares estimations) were used to evaluate the datasets. The coefficients of determination were tested with a two-sided test for significance of the regressions. Hypothesis testing on equal means of groups or on parity of the mean of the population and a specified value was carried out using Student's *t*-test (two-sided). This was performed against a 95% confidence interval, preceded by a test for normal distribution. All procedures followed standard protocols (Sachs, 1984).

3. Results

3.1. Pasture and feed

The mean sward height during the experiment was approximately 6.2 cm (SD 0.9 cm, $n = 8$; Table 2). Sward height was closely correlated with forage composition (*e.g.*, with crude protein content, $r^2 = 0.74$) even though variation in sward height was small (range 5.5–6.9 cm). AIA content of herbage was 13.4 g/kg (SD 1.7 g/kg; $n = 8$). Due to beneficial conditions for herbage growth, sward height increased in weeks 2–3. Accordingly, the crude protein content decreased from 216 g/kg to 189 g/kg (Table 2). In the following period, protein content increased again, partially due to the excess harvesting of 15% of the pasture at the end of week 3 and the previous N fertilisation. After this intervention, the dry matter composition, eIOM and the mean sward height rapidly regained the levels seen in week 1.

3.2. Digestion experiment with wethers

The diet supplemented with grain maize had a significantly (P<0.05) higher $OMD_{in\ vivo}$ than the pure herbage diet (Table 3). The higher $OMD_{in\ vivo}$ of the supplemented diet was due to a significantly (P<0.001) higher digestibility of the NFC fraction, which is reasonable as supplemented grain maize mainly contributes to this fraction. The significantly (P<0.05) higher $OMD_{in\ vivo}$ in the ADF_{om} fraction in the grain-maize supplemented group could be caused by a better energy supply of cellulolytic bacteria in the rumen. AIA content in faeces of wethers was 59.5 g/kg for the grain-maize supplemented group and 57.0 g/kg for the pure herbage group.

3.3. Cow performance

There were no clinical signs of diseases in either of the two groups during the entire experiment. The DM intake of herbage (15.5 kg/d *vs.* 15.8 kg/d) and grain maize (1.72 kg/d *vs.* 1.68 kg/d) did not differ significantly between the two groups

Table 4
Energy-corrected milk (ECM[a]), body weight, body condition score and back fat thickness during the two experimental periods. Each mean is calculated from 84 (ECM), 24 (body weight), 4 (body condition score) and 4 (back fat thickness) replicated measurements.

Item	Period 1				Period 2			
	Pasture group P-S		Stall group S-P		Pasture group S-P		Stall group P-S	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
ECM (kg/d)	25.0	0.6	26.2	1.1	22.5	0.3	23.0	0.3
Body weight (kg)	649	56	623	52	619	52	663	58
Body condition score	3.31	0.24	3.31	0.29	3.13	0.48	3.31	0.13
Back fat thickness (cm)	1.02	0.26	0.95	0.18	0.91	0.17	1.11	0.30

[a] ECM, energy-corrected milk, ECM = (0.3246 × kilograms of milk) + (12.86 × kilograms of milk fat) + (7.04 × kilograms of milk protein).

in the stall. Milk production, BW, body condition score and back fat thickness throughout the experiment (Table 4) remained similar between both groups whether pasture- or stall-fed. The initial average ECM of 28 kg per day decreased slightly to 25 kg/d on the pasture and in the stall during the first three weeks of period 1 (Table 5), in agreement with other authors (Soriano et al., 2000). This development related to changes in feed properties and the progress in lactation.

3.4. Cow faeces

The crude protein content in faeces OM averaged 214 g/kg (SD 23 g/kg; $n=48$) with significantly ($P<0.001$) lower crude protein contents in the stall (199 g/kg; SD 17 g/kg; $n=24$) than on pasture (229 g/kg; SD 17 g/kg; $n=24$). In contrast, the difference between group S-P and group P-S was small and not significant (209 g/kg; SD 19 g/kg; $n=24$ *vs.* 219 g/kg; SD 26 g/kg; $n=24$). During stall feeding, the crude protein content decreased substantially from 224 to 178 g/kg during weeks 2 and 3 of period 1. This change probably reflected the change in sward properties (Table 2). In contrast, no strong decline was apparent during this time on the pasture, perhaps due to some selection by the cows, which compensated for changing sward properties. During the remaining period of the experiment, the N content in the faeces in the stall and on the pasture was more constant. The AIA content in faeces was 69 g/kg (SD 4 g/kg; $n=24$) for grazing cows and 64 g/kg (SD 4 g/kg; $n=24$) in the stall.

The Ti content in the faeces DM during the experiment varied little and was on average 3.78 g/kg (SD 0.5 g/kg; $n=48$). A rather small difference was noted between the two experimental groups (3.82 g/kg for group S-P and 3.75 g/kg for group P-S). No significant difference was observed between the cows on the pasture and the cows in the stall. The lower N content in faeces during week 2 and 3 of period 1 was, therefore, not associated with a lower Ti content. This meant that ingestion on the pasture should have been greater than in the stall. However, no further evidence for this (*e.g.* in terms of higher milk yields) was noted, although the effect of higher intake should have been amplified by the higher digestibility of herbage on the pasture.

3.5. Comparison among the digestibility methods

OMD_{N1} (valid for fresh herbage) was 0.76 when averaged for all faeces samples, almost the same as that of OMD_{N2} (0.75) and OMD_{N3BH} (0.75) (Fig. 1). This similarity was based on the corresponding equations, which predicted very similar OMD within the range of faecal N contents occurring in the experiment (Fig. 2). However OMD_{N3G} (0.68) deviated considerably, without any obvious reason why the equation for OMD_{N3G} should have been less applicable than that for OMD_{N2} or OMD_{N3BH}. The N content of faeces, on which these calculations are based, differed significantly between stall- and pasture-fed animals ($P<0.05$). All three equations predicted that OMD on pasture was higher by about 0.02 (Fig. 1). The difference between group S-P and P-S was small (about 0.005). The mean variation within each group was 0.015 (SD).

OMD_{AIA} for stall-fed cows was significantly higher than estimated by the other methods (0.80). In the wether experiments $OMD_{in\ vivo}$ was, with the exception of the AIA method, consistently higher than estimated by the other methods (by 0.02–0.11; compare Figs. 1 and 3). $OMD_{in\ vivo}$ was 0.79 for the herbage-maize diet and 0.78 for the pure herbage diet. $OMD_{in\ vitro}$ for

Table 5
Energy-corrected milk (ECM), feed intake and digestibility of organic matter (OMD) of ingested feed measured with the faecal N method for fresh herbage (OMD_{N1}) throughout the experimental period. Means are calculated from 28 milk and feed intake measurements or 4 digestibility measurements. Period '0' denotes the week of acclimatisation after an experimental change. SD is the standard deviation of replicates within each cell averaged over all weeks.

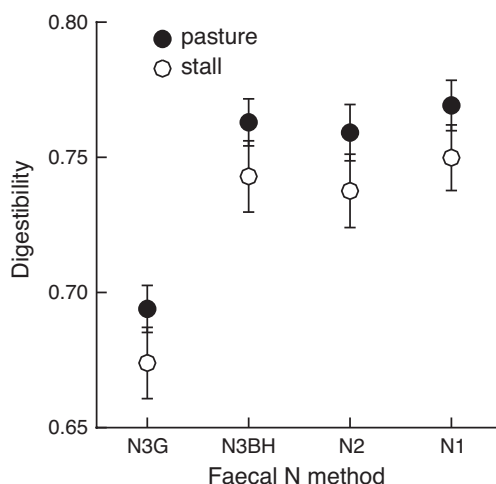| Week:	1	2	3	4	5	6	7	8	SD
Period:	0	1	1	1	0	2	2	2	
ECM in stall (kg/d)	28.9	27.2	26.4	25.1	22.5	22.8	22.5	22.2	2.6
ECM on pasture (kg/d)	27.2	25.6	24.8	24.5	25.3	23.1	22.7	23.3	1.5
Feed intake in stall (kg/d)		16.1	15.7	14.8		15.8	16.1	14.8	1.2
OMD in stall		0.767	0.752	0.734		0.749	0.742	0.755	0.007
OMD on pasture		0.782	0.775	0.771		0.761	0.763	0.763	0.005

Fig. 1. Digestibility of organic matter estimated by different faecal N methods. Error bars indicate standard deviation, which is caused by the variation in N content that is identical for all methods. N1, faecal N method for fresh herbage (OMD_{N1}); N2, faecal N method for conserved forage-based diets (OMD_{N2}); N3BH, faecal crude protein method for Braunschweig and Hohenheim (OMD_{N3BH}); N3G, faecal crude protein method for Gumpenstein (OMD_{N3G}).

pure herbage yielded 0.73 on average. The low level of $OMD_{in\ vitro}$ was due to the low values in weeks 2 and 3 (0.69 and 0.67). $OMD_{in\ vitro}$ was thus similar to the digestibilities based on faecal N, except for OMD_{N3G}, considering the slightly higher digestibility of the herbage-maize diet when compared to pure herbage (Table 3).

The AIA method and the Ti method were applicable only in the stall (group S-P in period 1 and group P-S in period 2), because of the need for measured feed intake. OMD_{Ti} was 0.75 and correlated significantly with OMD_{N1} (Fig. 4) and the other methods based on faecal N. For OMD_{N1}, the regression did not differ from the 1/1 line and was significant, but weak ($r^2 = 0.47$). Excluding one animal, causing three outliers, from the calculation increased r^2 to 0.57. The weak correlation does not imply that both methods correlate weakly in general. In the present case, the correlation was weak due to the experimental setup, which aimed at a constant herbage quality during the whole experiment. If a constant digestibility had been fully achieved, a zero correlation would have resulted with all variation due to experimental error. An r^2 of about 0.5 indicates that inaccuracies of the methods contributed about half to the variation (0.02) while the other half (0.02) was attributable to true variation due to the lower herbage quality in weeks 2–4.

4. Discussion

To our knowledge, this is the first study where herbage for stall-feeding was harvested twice daily between grazing animals on a pasture over a prolonged time. This method was developed to avoid problems of dissimilarities between grazed and mown swards and it delivered a sound basis for estimating OMD of herbage from pasture using different methods. The mown herbage for evaluation in the stall has to be near-identical to the grazed herbage, which makes the selection of an
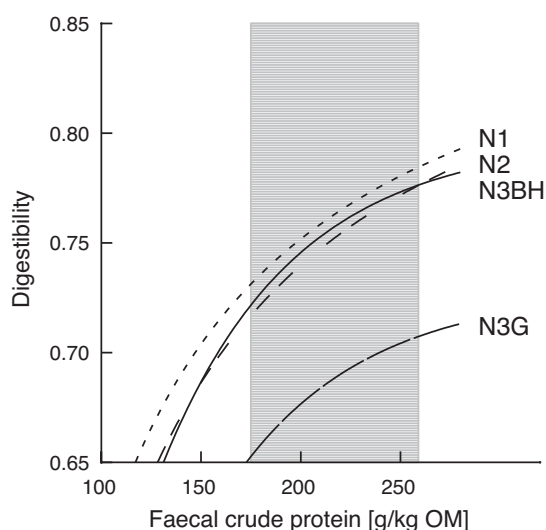

Fig. 2. Calibration curves of different faecal N methods. N1, faecal N method for fresh herbage; N2, faecal N method for conserved forage-based diets; N3BH, faecal crude protein method for Braunschweig und Hohenheim; N3G, faecal crude protein method for Gumpenstein. The grey area shows the range of our data.
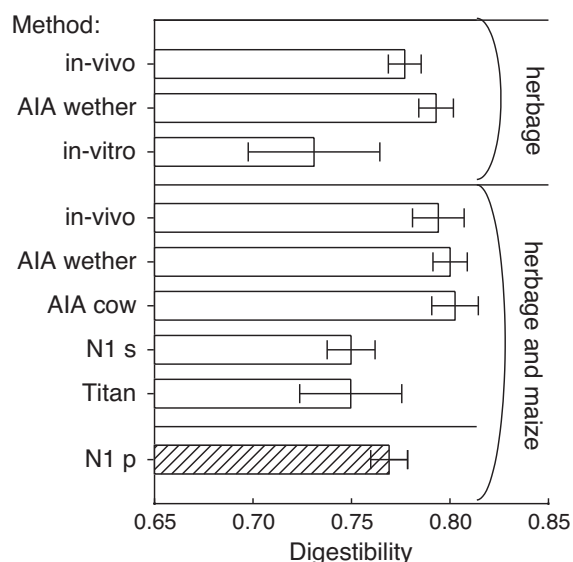
Fig. 3. Organic matter digestibility estimated by the wether test (*in vivo*), the acid-insoluble ash method (AIA); the pepsin-cellulase method (*in vitro*), the Ti method (Titan) and the faecal N method depending on feed (herbage *vs.* herbage + maize) and location of measurement (stall: open bars, pasture: hatched bars). Error bars indicate standard deviation.

appropriate mowing height critical. At high stocking densities the bite depth may vary between 45 mm (Meijs and Hoekstra, 1984; Mayne et al., 1990), 44 mm (O'Donovan and Delaby, 2008) down to 15 mm (Illius and Gordon, 1987). We chose 30 mm mowing height, which is within these values and which agreed with observations in a previous experiment.

Only the faecal-N based methods were entirely applicable to grazing animals. The OMD$_{in\ vitro}$ method should theoretically also work on pasture, given that a representative sampling is possible, but it excludes animal-specific effects. Methods using artificially supplied tracers, like Ti in our case, require that some supplements must be fed, in order to apply the tracer, but this may help to access the animal. In the case of small ruminants, the tracer may be supplied directly, thereby avoiding supplementary feed (Glindemann et al., 2009, in the case of sheep), but this involves disturbance of the animals and a large work load. Alternatively, a bolus with the respective marker can be injected. However, a constant release of the marker over a prolonged period must then to be ensured.

Internal tracers, like AIA in our case, depend on the precondition that material of exactly the same composition as the feed grazed by the animals can be obtained as a reference, which becomes especially difficult where feed from different sources can be selected by the animal. The small amounts of AIA, especially in herbage, where a relatively large variability can also be expected, requires sufficient material in order to control measuring errors.

N content in faeces of pasture- and stall-fed animals differed significantly in the present study, indicating a high sensitivity of the faecal-N methods to detect even small differences in OMD. Despite the higher OMD of grazed herbage, milk yield was
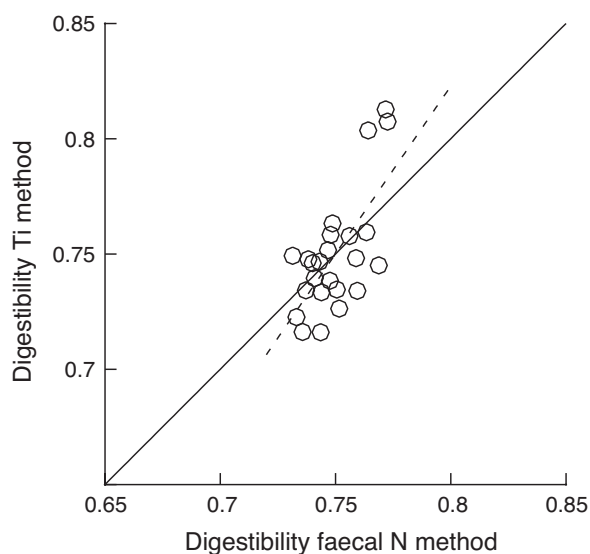

Fig. 4. Relation between estimates of organic matter digestibility by the faecal N method (OMD$_{N1}$) and the Ti method (OMD$_{Ti}$) for individual animals. The solid line denotes unity. The regression (dashed line) is $y = 1.455x - 0.342$, $r^2 = 0.47$.

slightly lower for grazing animals. This might be due to the energy demand of locomotion when grazing on pasture with low sward heights.

Both, the difference in milk yield and the difference in N content, indicate that even with a considerable effort to obtain the feed for the stall it was not fully possible to simulate a grazing cow. Even with grazing to a sward height of 6.2 cm and additional mowing every 10 days, some selection must still have occurred. The difference between grazed feed and sampled (mown) feed quite likely increases when the grazing systems allows for more selection or where the feed sample is obtained from outside the grazed area akin in the case of exclosure cages. Exclosures next to the experimental pasture are often used to harvest herbage and to calculate DM intake and digestibility (Polan et al., 1986; Holden et al., 1994), especially when a control group is fed with herbage in the stall. The time of exclosure has to be sufficiently long to produce a clear difference between exclosure and the grazed area (*e.g.* one month in the experiments of Wittmer et al. (2009) and Schiborra et al. (2010)) as the DM intake is given by the difference between pre- and post-grazing biomass. The results of period 1 clearly show that, even under grazing, which should retard aging in the growing sward, the quality of feed can change within two weeks. This calls for a different approach.

Tracer methods require an assumption about the recovery rate. For Ti, Titgemeyer et al. (2001) measured a recovery rate of 900–950 g/kg and 930 g/kg in the case of cattle fed on forage-based diets. Hafez et al. (1988) observed 960–1.020 g/kg faecal recovery in dairy cows fed concentrate, grass-silage and corn-silage diets, although diurnal variation in excretion pattern was high. On average, for theses studies recovery rate was 960 g/kg, which was assumed in this study. The recovery rate is especially critical for artificial tracers, which are applied for only a specific time period because full flow equilibrium may not have been achieved. In our case, the time course of Ti excretion provided no indication that flow equilibrium had not been achieved.

Faecal N methods are not affected by these uncertainties. These methods, however, suffer from other weaknesses. Their sensitivity is small when the digestibility is low, but with a grazing system providing feed of very high digestibility, as in our case, the sensitivity becomes high as indicated by the slope increasing with digestibility until the maximum digestibility inherent in a certain equation is reached (Fig. 2). $OMD_{in vivo}$, $OMD_{AIA\,wether}$ and $OMD_{AIA\,cow}$ all indicated that digestibility was 0.80 on average and higher in some cases (Fig. 3). Even OMD_{Ti}, which presumably underrated digestibility due to overestimated recovery rate, partially produced digestibilities well above 0.80 (Fig. 3). For OMD_{N3G} and OMD_{N3BH} digestibility already exceeded the maximum digestibilities (0.73 and 0.79) and thus must underrate digestibility in our case. Deviations in N content to lower values will lead to low digestibilities, while deviations to higher values cannot exceed the OM threshold in this case. Digestibilities over 0.80 can only be obtained with OMD_{N1} and OMD_{N2}. Calculating the digestibility from the average N content led to 1.5 g/kg higher digestibilities than did averaging the digestibilities as calculated from the individual N-faeces measurements. The faecal-N methods that are presently used suffer from a further and major disadvantage: the regression models to calculate OMD need a calibration and are only suitable for a particular measurement range.

OMD_{N1} and OMD_{N2} have no location factor and in our case delivered results that agreed with OMD_{Ti}. The main source of error arising during calculation of OMD with the faecal-N methods was the selection of an appropriate equation that included the location factor "a_i" in Eq. (6). For Braunschweig and Hohenheim (OMD_{N3BH}), the results agreed with the other faecal-N methods, whereas OMD_{N3G} significantly deviated. Lukas et al. (2005) favoured using OMD_{N3BH} for nearly all conserved foods and this was confirmed by studies of Schlecht and Susenbeth (2006), Schiborra et al. (2010) and Wang et al. (2009). The reason for the difference in location factors between Braunschweig/Hohenheim and Gumpenstein is unclear.

5. Conclusions

At present no unambiguously valid method exists for determining digestibility of feed grazed at pasture. The methods based on the N content in faeces have the largest potential because they are easily measured and the method does not rely on assumptions such as appropriate feed sampling or tracer recovery rate. The faecal-N methods (OMD_{N1} and OMD_{N2}) are suitable for highly digestible feed such as herbage from all-day pasture, where their sensitivity is good. The faecal-N methods also appear to be well suited for a wide range of experiments because of their low costs and methodological simplicity.

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