SHORT COMMUNICATION

Comparisons of methods for in vitro dry matter digestibility of ruminant feeds

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[1]*Animal Nutrition Group, Department of Animal Science, Wageningen University, 6700 AH Wageningen, the Netherlands; and* [2]*Department of Animal Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2.* *Received 19 October 2004, accepted 21 February 2005.*

Brons, E. and Plaizier, J. C. 2005. **Comparisons of methods for in vitro dry matter digestibility of ruminant feeds.** Can. J. Anim. Sci. **85**: 243–245. Apparent in vitro dry matter digestibilities of selected ruminant feeds were determined with the DAISY^{II} incubator (ADD, ANKOM Technology Corp., Macedon, NY) and the Tilley and Terry technique (ADTT). True in vitro dry matter digestibility was also determined with the DAISY^{II} incubator (TDD). The ADD and ADTT did not differ for grain crop silages and total mixed rations. The ADD was 9.0 percentage points higher than ADTT for grains and 3.4 percentage points lower than ADTT for grass and legume forages. The TDD was between 5.7 and 11.2 percentage points higher than ADD depending on the feed.

Key words: In vitro dry matter digestibility, forages, grains, ANKOM DAISY^{II} incubator

Brons, E. et Plaizier, J. C. 2005. **Comparaison des méthodes de détermination de la digestibilité *in vitro* de la matière sèche des aliments pour ruminants**. Can. J. Anim. Sci. **85**: 243–245. Les auteurs ont déterminé la digestibilité *in vitro* apparente de la matière sèche de certains aliments pour ruminants au moyen de l'incubateur DAISY^{II} (ADD, ANKOM Technology Corp., Macedon, NY) et de la technique Tilley et Terry (ADTT). Ils se sont aussi servis de l'incubateur DAISY^{II} pour établir la digestibilité *in vitro* véritable de la matière sèche (TDD). L'ADD et l'ADTT ne varient pas pour les ensilages de grain et la ration mixte totale. L'ADD a 9,0 points de pourcentage de plus que l'ADTT pour les grains et 3,4 points de pourcentage de moins que l'ADTT pour les fourrages de graminées et de légumineuses. La TDD se situe entre 5,7 et 11,2 points de pourcentage au-dessus de l'ADD selon l'aliment.

Mots clés: Digestibilité *in vitro* de la matière sèche, fourrages, grains, incubateur DAISY^{II} d'ANKOM

The Tilley and Terry technique for the determination of apparent in vitro dry matter (DM) digestibility [(ADTT) Tilley and Terry 1963; Goering and van Soest 1970)] has been used extensively with ruminant feeds. This technique consists of two stages: incubation with rumen inoculum and buffer followed by incubation with acid and pepsin to digest the protein that remained undigested during the first incubation (Tilley and Terry 1963). Goering and Van Soest (1970) refer to this digestibility as apparent, since the residue may contain bacterial residues and other pepsin-insoluble materials as well as undigestible fibre. True in vitro DM digestibility can be determined by measurement of the neutral detergent fibre (NDF) in the residue from the incubation with rumen inoculum and buffer (Goering and Van Soest 1970).

In order to increase labour efficiency and the precision of the determination of in vitro DM digestibility, the DAISY^{II} incubator (ANKOM Technology Corp., Macedon, NY) was developed. This incubator allows incubation of up to 100 samples at a time in filter bags. Holden (1999) and Mabjeesh et al. (2000) compared the ADTT with in vitro DM digestibilities obtained by a two-step incubation, i.e., simulated rumen digestion followed by simulated gastric digestion with acid and pepsin using the DAISY^{II} incubator (ADD). Holden (1999) did not observe significant differences between the ADD and ADTT of 10 test feeds that included forages, concentrates and total mixed rations (TMR). Mabjeesh et al. (2000) found that ADD and ADTT were similar for forages, corn grain, soybean meal, and corn gluten feed, but that ADD was higher than ADTT for barley grain ($P < 0.02$), wheat grain ($P < 0.01$), sunflower meal ($P < 0.001$), rapeseed meal ($P < 0.001$) and fish meal ($P < 0.001$). In their study, the R^2 of the regression of ADD on ADTT was 0.81, but when feeds that had higher ADD than ADTT were excluded, the R^2 increased to 0.95. Mabjeesh et al. (2000) concluded from these results that the DAISY^{II} method can be used to predict in vitro DM digestibility of ruminant feeds.

ANKOM Technology (2004) recommends determining true in vitro digestibility with the DAISY^{II} incubator (TDD). To our knowledge differences between ADD and TDD have not been quantified using ruminant feeds. The objectives of this study were to compare ADD and ADTT and to quantify differences between ADD and TDD using feed samples collected on Manitoba dairy farms.

A total of 167 feed samples were collected on 40 randomly selected dairy farms in Manitoba. Samples included

Abbreviations: **ADTT**, apparent digestibility determined with Tilley and Terry technique; **ADD**, apparent digestibility determined with DAISY^{II} incubator; **DM**, dry matter; **NDF**, neutral detergent fibre; **TDD**, true digestibility determined with DAISY^{II} incubator; **TMR**, total mixed ration

grass/legume forages, grain crop silages, grains and total mixed rations (TMR). The number of samples in each group is given in Table 1. The DM of all feed samples was determined by drying at 60°C for 48 h (Association of Official Analytical Chemists 1990, 935.26). Samples were subsequently ground through a 1-mm screen in a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA). In vitro DM digestibilities of samples were measured using three techniques: a modified Tilley and Terry technique (Tilley and Terry 1963) to determine apparent digestibility (ADTT); incubation of sample feeds in filter bags in a DAISY II incubator (ANKOM Technology Corp., Macedon, NY) with rumen inoculum and buffer followed by acid digestion with pepsin to determine apparent digestibility (ADD); incubation of feed samples in filter bags in a DAISY II incubator with rumen inoculum and buffer followed by determination of NDF in the residue to determine true digestibility (TDD). All analyses were done in duplicate. If duplicates varied more than 5% the analysis was repeated.

The ADTT was determined as described by Tilley and Terry (1963). Approximately 500 mL of rumen fluid and particulate matter were obtained from a rumen-fistulated steer fed alfalfa hay and transported in a prewarmed insulated container. The inoculum was prepared by blending ruminal fluid and particulate matter for 5 to 8 min in a Waring blender under constant purging with CO_2, followed by filtering with vacuum through 5 to 8 mm of glass wool, wrapped in one layer of cheesecloth with constant purging with CO_2. Buffer solution was prepared by adding 9.8 g of $NaHCO_3$, 7.0 g of $Na_2HPO_4 \cdot 7H_2O$, 0.57 g KCl, 0.47 g NaCl, 0.04 g $CaCl_2$, 0.12 g $MgSO_4 \cdot 7H20$, 0.9161 g urea and 0.9169 g glucose (dextrose) in 1 L of deoinized water. Rumen inoculum and buffer solution were combined at a ratio of 2:3. Approximately 500 mg of each feed and 12.5 mL of the combined buffer and rumen inocolum solution were added to 50-mL glass tubes. The tubes were filled with CO_2 and a stopper was added. Samples were digested for 48 h at 39°C in an incubator with continuous shaking. Pepsin solution was prepared by dissolving 20 g of pepsin (Laboratory grade, Product P53-500, Fisher Scientific Fair Lawn, NJ) in 500 mL of 0.1 M HCl. Following the first incubation, 2.5 mL of pepsin solution and 2.5 mL of 1 M HCl were added to each tube. The pH of the incubated solution was reduced to 1.2, and tubes were purged with CO_2. After 24 h of incubation, tubes were removed from the incubator and the solution was filtered. The residue was washed three times with hot deionized water and twice with acetone. The DM content of the residue was determined by drying overnight at 100°C. Each run included two blank tubes to which no sample was added.

For both methods using the DAISY^{II} incubator, approximately 500 mg of each feed was placed in ANKOM F57 filter bags with a pore size of 25 µm (Ankom Technology Corp., Macedon, NY), which were subsequently heat-sealed. Two solutions were prepared (ANKOM Technology 2004). Solution A contained 10 g of KH_2PO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of NaCl, 0.1 g of $CaCl_2 \cdot 2H_2O$, and 0.5 g of urea in 1 L of deionized water. Solution B contained 15 g of Na_2CO_3 and 1 g of $Na_2S \cdot 9H_2O$ in 100 mL of deionized water. Both solutions were prewarmed and 20 mL of solution B was added to 1 L of solution A. The exact amount of A to B was adjusted to obtain a final pH of 6.8. A total of 1600 mL of buffer solution, 400 mL of rumen inoculum and 25 filter bags were added to each of the 4-L digestion vessel of the DAISY^{II} incubator. Vessels were subsequently flushed with CO_2 and placed in the DAISY^{II} incubator at 39°C for 48 h (ANKOM Technology 2004) under continuous rotation.

For the determination of ADD, 8 g of pepsin and 50 mL of hydrochloric acid were added to each incubation vessel after the first incubation, and incubation was continued for another 24 h (Holden 1999). After the second incubation, incubation vessels were removed, fluid was drained, and the filter bags were rinsed with cold tap water. Subsequently, the filter bags were washed in with water at 90–100°C, washed with acetone and dried overnight in an oven at approximately 100°C.

The first incubation for the determination of TDD was identical to that for ADD. After the first incubation, the incubation vessels were removed and the fluid was drained. Filter bags were rinsed with cold tap water and frozen until NDF determination. NDF was determined according to Van Soest et al. (1991) by incubating the filter bags in an ANKOM^{200} Fiber Analyzer (ANKOM Technology Corp., Macedon, NY) at neutral pH with sodium lauryl sulphate and alpha-amylase. For the determination of ADD and TDD, one heat-sealed filter bag to which no feed sample was added was included in each incubation vessel.

Data were analyzed with the MIXED procedure of the SAS Institute Inc. (1990). The effects of method and feed group and their interaction were considered fixed. The effect of sample within feed group was considered random. Statistical significance was set at a P value equal to or less than 0.05. As the interactions between method and feed group were significant, analyses were conducted by feed group. Regressions between ADTT and ADD and between ADD and TDD were determined with the general linear models procedure of the SAS Institute Inc. (1990).

Averages and SD of ADTT, ADD and TDD for feed groups are given in Table 1. The ADTT and ADD of grass/legume forages were, respectively, 8.9 and 4.4 percentage points higher than the values obtained by Holden (1999) and, respectively, 7.8 and 5.7 percentage points higher than the values obtained by Mabjeesh et al. (2000). On average, ADTT and ADD of grain crop silages were, respectively, 2.7 and 2.0 percentage points lower than the values obtained by Holden (1999). The ADTT and ADD of grains were, respectively, 2.6 percentage points lower and 1.5 percentage points higher than the values obtained by Holden (1999) and, respectively, 2.5 percentage points lower and 1.9 percentage points higher than the values obtained by Mabjeesh et al. (2000). On average, ADTT and ADD of TMR were, respectively, 1.5 and 0.5 percentage points higher than the values obtained by Holden (1999). Apparent digestibilities of grain crop silages, TMR, and grains determined by Holden (1999), Mabjeesh et al. (2001) and the current study are comparable. The higher apparent digestibilities

Table 1. Mean in vitro DM digestibility (percentage) for apparent in vitro digestibilities determined with the Tilley and Terry method (ATT) and incubation in the DAISY^{II} incubator (ADD, ANKOM Technology Corporation Macedon, NY) and true in vitro digestibility determined with the DAISY^{II} incubator (TDD) for selected ruminant feeds

	Method					Effects (*P* value)	
Feed group	ADTT	ADD	TDD	SE	N	ADD vs. ADTT	ADD vs. TDD
Grass/legume forages	63.4	60.0	70.7	0.45	76	<0.0001	<0.0001
Grain crop silages	61.2	60.9	72.1	0.67	35	NS[z]	<0.0001
Grains	77.6	86.6	92.3	0.74	25	<0.0001	<0.0001
Total mixed rations	70.4	69.0	79.1	0.44	31	NS	<0.0001

[z]NS = not significant

of grass/legume forages in the current study compared to the earlier studies could be due to differences in the grass/legume forages among studies, as apparent digestibility of grain crop silages and TMR showed much less variation among studies.

The ADD and ADTT did not differ for grain crop silages and total mixed rations. The ADD was 3.4 percentage points lower ($P < 0.0001$) than ADTT for grass/legume forages. This agrees with Holden (1999) who found no difference in in vitro DM digestibilities of forages and TMR between these techniques. Mabjeesh et al. (2000) also concluded that for forages both techniques resulted in comparable in vitro DM digestibilities. For grains, ADD was 9.0 percentage points ($P < 0.0001$) higher than ADTT. The same was observed by Mabjeesh et al. (2000) for barley grain and wheat grain. Holden (1999) also found that for corn grain, ADD was numerically higher than ADTT. These differences between the two techniques were explained by the fact that grains become floury when ground and that not all feed particles passing through the 50 μm pores of the filtration bags are digested by microbial enzymes and pepsin.

Averaged across forages, TDD was 8.4 and 10.4 percentage points higher ($P < 0.05$) than ADTT and ADD, respectively. For TMR, TDD was 8.7 and 10.1 percentage points higher ($P < 0.05$) than ADTT and ADD, respectively. The ADTT did not differ from the ADD for corn silage, oatlage, barley silage, straw and TMR, but was higher than ADD for mixed and alfalfa hays and mixed and alfalfa silages. For corn grain and barley grain, TDD were also higher than ADTT and ADD, and ADTT were lower than ADD. For wheat grain, similar differences were observed, but they were not significant.

Regression of ADD on ADTT and of ADD on TDD across all feeds resulted in R^2 of 0.81 and 0.91, respectively. Mabjeesh et al. (2000) also obtained an R^2 of 0.81 for the regression of ADD on ADTT across feed groups.

This study showed that apparent in vitro DM digestibilities of grass/legume forages, grain crop silages, and TMR determined using the DAISY^{II} incubator are comparable with those determined using the Tilley and Terry method. Results suggest that apparent in vitro DM digestibilities of grains determined with the DAISY^{II} incubator overestimate DM digestibility. For these feeds, ADTT might be more accurate than ADD for the determination of in vitro DM digestibility.

Kathryn Suraski, Sharon Pydee, Terri Garner, and Janice Haines are thanked for their technical assistance. This study was supported by grants from Dairy Farmers of Manitoba, the Manitoba Rural Adaptation Council (MRAC) and the Dairy Farmers of Manitoba.

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