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# A note on the evaluation of the acid-insoluble ash technique as a method for determining apparent diet digestibility in beef cattle

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**The objective was to determine if the acid-insoluble ash (AIA) method provided accurate estimates of *in vivo* apparent digestibility compared with the standard total faecal collection (TFC) method. Twelve steers, mean live weight 328 (s.d. 27.3) kg, were offered one of three diets based on whole-crop wheat (WCW) or a grass silage (GS) diet in a 4 × 4 latin square design. Apparent dietary digestibility was determined simultaneously using AIA and TFC methods. Agreement between the two methods depended on diet type, with acceptable agreement (a difference between the methods of 0.06), observed with the WCW-based diets. However, the strength of the agreement was weakened with the inclusion of GS. Agreement statistics were found to be a useful tool for assessing the relationship between the two methods of measurement.**

*Keywords*: acid-insoluble ash; cattle; digestibility; total faecal collection

## Introduction

Quantification of the digestibility of dietary components is helpful in understanding differences in the nutritive values among ruminant diets. The total faecal collection (TFC) method has been the method used most commonly for the determination of *in vivo* diet digestibility. However, labour availability and the specialised animal handling facilities required for the TFC method can restrict its use. This has prompted the investigation of methods based on indigestible internal and external markers as an alternative. External markers include ferric oxide and chromic oxide, but diurnal patterns of excretion

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and unreliable adherence to feed particles may negatively impact on their accuracy (Thonney *et al*., 1985). These issues are not evident with internal markers, such as acid-insoluble ash (AIA), silica, indigestible neutral detergent fibre (NDF) and indigestible acid detergent fibre (ADF) and these have the potential to provide reliable estimates of digestibility (Thonney *et al*., 1985). In addition, livestock do not have to be confined in specialised digestibility measurement facilities when using these markers, and there is minimal disruption of herd behaviour and other animal activities (e.g., feeding, milking). Thus, digestibility can be measured under normal conditions of animal production. In a review of the AIA method, Sales and Janssens (2003) reported strong relationships with the TFC method, although many of the reviewed studies were carried out in non-ruminant species. The present study aimed to determine if the AIA method provided accurate estimates of apparent dietary digestibility in cattle offered diets based on whole-crop wheat (WCW) silage or grass silage (GS).

## Materials and Methods

Twelve Holstein-Friesian steers, mean live weight 328 (s.d. 27.3) kg, were assigned to one of three blocks on a descending live weight basis and randomly allocated, from within block, to one of four diets based on either WCW or grass silage in a 4 × 4 latin square design. The WCW was grown, harvested and processed as described by McGeough *et al*. (2010b). Three of the diets were based on WCW and involved three ratios (on a dry matter basis) of WCW grain to straw plus chaff as follows: 11:89 (WCW I), 26:74 (WCW II) and 47:53 (WCW III). The fourth treatment was based on GS.

Each period within the latin square consisted of 18 days adaptation and 10 days for measurement of digestibility. All silages were offered, *ad libitum,* in a single feed daily for 15 days. The amount of silage offered to each animal was restricted to 0.90 of its average *ad libitum* intake during the final 3 days of the adaptation period (Owens *et al*., 2009). A concentrate supplement (2.60 kg DM) was offered to each animal daily in a single feed for the first 15 days, and then at a rate such that the concentrate to silage ratio was equivalent to that of the mean of the animals on the corresponding treatment during the preceding week in a concurrent animal production study (Mc Geough *et al*., 2010b). Animals were offered their respective diets at 1030 daily, and had continuous access to a clean fresh water supply. Diet digestibility measurements were undertaken, using the TFC method, for the 10 days following adaptation. Faecal collection and sampling were carried out at 0800, as described by Moloney and O'Kiely (1997). The offered silages and concentrate were sampled daily during the faecal collection period and composited.

Simultaneously with faecal collection diet digestibility was determined for all animals using the AIA method, as described by Van Keulen and Young (1977). Faecal grab samples (200 g) were obtained daily (at 0800) from, per rectum, and pooled for each animal at the end of the measurement period. All feed and faecal samples were processed and subsequently assayed according as described by Mc Geough *et al*. (2010a).

The two methods were compared using graphical representation on an agreement plot and by computing a total deviation index (TDI) and coverage probability (CP) (Lin *et al*., 2002). Computation was carried out using the SAS macro of Lin *et al*. (2002), available at http://www.uic.edu/~hedayat/, with a constant variance and the TFC method treated as a random variable. An acceptable level of agreement was specified as a difference between the methods of 0.06 with a CP of 90%. However, this analysis was not done for starch digestibility as there was insufficient variation between and within the treatment groups. Relationships among digestibility estimates were also assessed by regression using Proc GLM (SAS, 2002–2003).

## Results

The mean chemical composition of the WCW and grass silages and the concentrate supplement are presented in Table 1. The digestibility estimates, obtained by the AIA and TFC methods, are presented in Table 2. The $TDI_{0.9}$, which is an estimate of the deviation that covers 90% of the digestibility measurements, was 0.08 for apparent DM digestibility (Table 3) and the $CP_{0.06}$ estimate was 0.75, which indicates that 75% of the observations fell within the previously specified error limit of 0.06. The $TDI_{0.9}$ and $CP_{0.06}$ for NDF digestibility were 0.12 and 0.58, respectively, and for crude protein were 0.09 and 0.72, respectively. However, upon removal of GS from the analysis, the strength of the agreement between the AIA and TFC methods increased as evidenced by

**Table 1. Composition of the whole-crop wheat silage, grass silage and concentrate supplement (mean ± s.d.)**

| Component | Whole-crop wheat silage | | | Grass silage | Concentrate supplement |
|---|---|---|---|---|---|
| | I | II | III | | |
| Dry matter (g/kg) | 440 ± 18.7 | 527 ± 24.0 | 572 ± 12.6 | 249 ± 9.1 | 869 ± 7.6 |
| Chemical composition (g/kg DM) | | | | | |
| Acid-insoluble ash | 17.7 ± 1.94 | 13.0 ± 3.22 | 9.0 ± 2.21 | 5.8 ± 0.84 | 3.2 ± 1.26 |
| Starch | 205 ± 25.7 | 332 ± 20.6 | 454 ± 26.7 | ND[1] | 246 ± 20.7 |
| Neutral detergent fibre | 516 ± 18.8 | 397 ± 12.2 | 302 ± 21.5 | 509 ± 22.9 | 137 ± 7.6 |
| Acid detergent fibre | 308 ± 13.8 | 229 ± 12.4 | 169 ± 12.7 | 313 ± 18.0 | 70 ± 11.7 |
| Crude protein | 104 ± 2.0 | 107 ± 2.1 | 115 ± 2.6 | 138 ± 5.9 | 302 ± 10.6 |
| Ash | 53.4 ± 2.12 | 44.6 ± 2.60 | 36.4 ± 2.44 | 93.4 ± 3.60 | 73.7 ± 3.01 |

[1] Not determined.

**Table 2. Digestibility estimates (kg/kg) for diets based on whole-crop wheat silage and grass silage determined using two methods (mean ± s.d.)**

| Component | Whole-crop wheat silage[3] | | | Grass silage |
|---|---|---|---|---|
| | I | II | III | |
| AIA[1] method | | | | |
| Dry matter | 0.67 ± 0.028 | 0.70 ± 0.034 | 0.74 ± 0.039 | 0.87 ± 0.017 |
| Neutral detergent fibre | 0.47 ± 0.042 | 0.42 ± 0.067 | 0.41 ± 0.019 | 0.76 ± 0.019 |
| Crude protein | 0.70 ± 0.028 | 0.69 ± 0.033 | 0.70 ± 0.044 | 0.84 ± 0.021 |
| Starch | 0.99 ± 0.003 | 0.99 ± 0.002 | 0.99 ± 0.004 | 0.99 ± 0.004 |
| TFC[2] method | | | | |
| Dry matter | 0.65 ± 0.043 | 0.72 ± 0.024 | 0.75 ± 0.024 | 0.79 ± 0.020 |
| Neutral detergent fibre | 0.46 ± 0.031 | 0.45 ± 0.059 | 0.43 ± 0.095 | 0.76 ± 0.026 |
| Crude protein | 0.68 ± 0.040 | 0.70 ± 0.030 | 0.71 ± 0.034 | 0.76 ± 0.023 |
| Starch | 0.99 ± 0.001 | 0.99 ± 0.002 | 0.99 ± 0.004 | 0.98 ± 0.006 |

[1] Acid-insoluble ash.
[2] Total faecal collection.
[3] See Table 1.

**Table 3. Agreement statistics between the acid-insoluble ash and the total faecal collection methods for digestibility of dry matter, neutral detergent fibre and crude protein**

| Component[1] | Agreement statistic[2] | Data set[3] | |
|---|---|---|---|
| | | All | WCW |
| Dry matter | $TDI_{0.9}$ | 0.08 (0.10) | 0.06 (0.07) |
| | $CP_{0.06}$ | 0.75 (0.66) | 0.89 (0.80) |
| NDF | $TDI_{0.9}$ | 0.12 (0.14) | 0.11 (0.13) |
| | $CP_{0.06}$ | 0.58 (0.49) | 0.63 (0.53) |
| CP | $TDI_{0.9}$ | 0.09 (0.11) | 0.06 (0.08) |
| | $CP_{0.06}$ | 0.72 (0.63) | 0.87 (0.77) |

[1] NDF = neutral detergent fibre, CP = crude protein.

[2] $TDI_{0.9}$ = total deviation index that contains 90% of the observed differences, $CP_{0.06}$ = coverage probability of 90% for a true difference in digestibility of 0.06.

[3] All = data for whole-crop wheat silages and grass silage, WCW = data for whole-crop wheat silages only.

the numerically lower $TDI_{0.9}$ and higher $CP_{0.06}$ for each component (Table 3). The observed regression relationships between the results from the two methods are in Table 4 and the agreement plots are in Figure 1.

## Discussion

The apparent DM and crude protein digestibility for the WCW diets were similar to those reported by Walsh *et al.* (2009) for WCW silages differing in grain concentration. However, the lower NDF digestibility in the present study is most likely due to supplementary concentrates being offered, which reduces rumen pH and thus inhibits the activity of the cellulolytic bacteria (Grant and Mertens, 1992), thereby reducing fibre digestibility.

In the present study acceptable agreement was observed between the AIA and standard TFC methods, given the proportion of measurements that fell within the pre-specified range of acceptability, when data from all four treatments were included in the analysis. Thus, it may be concluded that the AIA method is useful when ranking the digestibility estimates for crops similar to those used here. However, results generated by the AIA method may not provide sufficiently accurate estimates of the values determined using the TFC method. The agreement plots demonstrate that the GS results were biased while the other results were generally scattered around the line of perfect agreement. The strength of the agreement in the present study was increased following the omission of data for GS.

The weaker agreement observed between the methods when GS was included may have been due to soil contamination in the grass silage (Van Keulen and Young, 1977), which is most likely to have occurred during harvesting. The

**Table 4. Regression relationship between the digestibility estimates obtained by the TFC and AIA methods[1]**

| Data set[2] | Component | Intercept (s.e.) | Slope (s.e.) | $r^2$ | P-value |
|---|---|---|---|---|---|
| All | Dry matter | –0.08 (0.087) | 1.13 (0.119) | 0.66 | <0.001 |
| | Neutral detergent fibre | –0.10 (0.033) | 1.23 (0.062) | 0.90 | <0.001 |
| | Crude protein | –0.12 (0.119) | 1.19 (0.166) | 0.53 | <0.001 |
| WCW | Dry matter | 0.28 (0.075) | 0.61 (0.106) | 0.49 | <0.001 |
| | Neutral detergent fibre | 0.05 (0.076) | 0.88 (0.170) | 0.44 | <0.001 |
| | Crude protein | 0.38 (0.109) | 0.45 (0.156) | 0.20 | <0.01 |

[1] Values by the total faecal collection (TFC) method as independent variable and those from the method based on acid insoluble ash (AIA) as dependent variable.

[2] See footnotes Table 3.

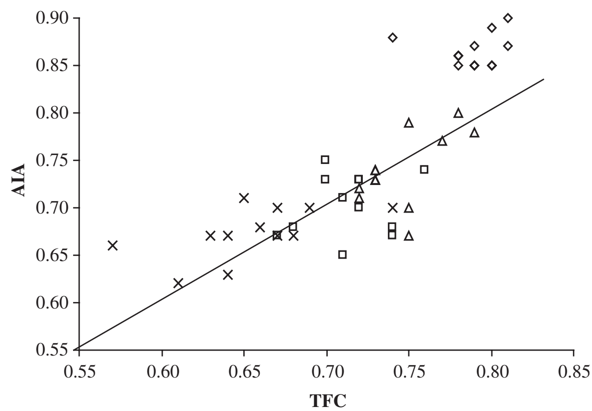


*Figure 1. Agreement between digestibility estimates, obtained by the acid-insoluble ash (AIA) and the total faecal collection (TFC) methods, for three whole-crop wheat silages (WCW I WCW II WCW III) and a grass silage (GS), for: a) dry matter, b) neutral detergent fibre, and c) crude protein. (*× WCW I; □ WCW II; △ WCW III; ◇ GS *represents line of perfect agreement).*

generally lower cutting height for grass than for cereal crops during silage making may have facilitated the uptake of soil, and ultimately led to the ingestion of silica with the silage and its accumulation in the digestive tract. This has been reported to distort estimates of digestibility when using the AIA method (Van Keulen and Young, 1977).

The regression analysis showed that the slopes of the fitted lines were greater than one, reflecting the bias in the GS measurements at the high end of the data range. Regression relationships determined with the inclusion of the GS data could result in poor generalisation of the relationship beyond the current data set. When the regressions were re-fitted without the GS data, the slopes were not found to be any closer to one and the $r^2$ value was reduced. This is due to the removal of the high values for GS and might be mistakenly taken as indicating poorer agreement of the methods. Thus, while regression may be required for calibration of a new method, agreement statistics will play a part in making a method more generally applicable.

The relationship observed between the AIA and TFC methods was weaker than that reported by Nousiainen, Rinne and Huhtanen (2009) for whole-crop and grass-silage based diets. The difference is most likely due to the large data set in the study of Nousiainen *et al*. (2009), which would reduce sampling effects on the estimates. It should be noted that, even given the small data set in this study, agreement statistics facilitated identification of important sources of disagreement.

In conclusion, estimates of dietary digestibility obtained using the AIA method were in tolerable agreement with those obtained using the standard TFC method.

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