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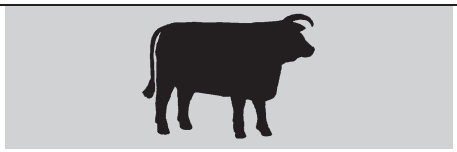

Case Study: Precision and Accuracy of Methodologies for Estimating In Vitro Digestibility of *Thinopyrum ponticum* (Tall Wheatgrass) Hay and Haylage Fed to Beef Cattle[1]

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ABSTRACT

Digestibility of feeds is a commonly used nutritive parameter and can be estimated through several techniques. The objective of the present study was to compare the precision and accuracy of different IVDMD techniques used to estimate in vivo DM digestibility (IV_{dig}) of tall wheatgrass hay and haylage (IV_{dig}: 42.2 to 53.3%). Forages were conserved after 1 of 3 regrowth periods (66, 96, and 162 d), generating a representative range of digestibilities, and were fed ad libitum to steers. The study analyzed goodness-of-fit for predictions of in vivo digestibility using in vitro apparent DM digestibility from Tilley and Terry ($T\&T_{dig}$) and a Daisy^{II} Incubator ($DAISY_{dig}$), and in vitro ruminal DM degradability using the gas-production technique (GAS_{deg}). In addition, 2 predictive equations from the literature were tested, Van Soest (VS_{dig}) and Rohweder (Ro_{dig}), based on fiber content of forage. The in vitro techniques showed higher correlations with IV_{dig} (R^2 = 0.97, 0.94, and 0.93, respectively) than VS_{dig} and Ro_{dig} (R^2 = 0.64 and 0.35, respectively). Biases were observed in all techniques, with correction factors of 0.34 for $T\&T_{dig}$, 0.74 for GAS_{deg}, and 0.88 for $DAISY_{dig}$. As a consequence, $DAISY_{dig}$ showed the greatest concordance (ρ_c = 0.85) compared with $T\&T_{dig}$ and GAS_{deg} (ρ_c 0.34 and 0.72, respectively), the predictive equations showed the poorest fits (ρ_c 0.21 and 0.33 for VS_{dig} and Ro_{dig}, respectively). Therefore, the recommended techniques are $DAISY_{dig}$ or GAS_{deg}, depending on requirements. However, all in vitro techniques showed biases, highlighting certain limitations for conserved forages.

Key words: conserved forage, in vivo digestibility, in vitro digestibility, *Thinopyrum ponticum*

INTRODUCTION

Digestibility is the most common nutritive parameter used in feeding standards for ruminants (Agricultural Research Council, 1980; Agricultural and Food Research Council, 1992; NRC, 1996; Coleman and Moore, 2003). Several laboratory techniques and predictive equations for digestibility exist in the literature, but despite their extensive use, the evidence suggests that their application to poor-quality forages has been relatively unsatisfactory or inconsistent between studies (Van Soest, 1994).

Tall wheatgrass (*Thinopyrum ponticum*) is a C_3 grass adapted to a wide range of environmental stressors, and is one of the most salt- and alkaline-tolerant cool-season forage grasses (Rogers and Bailey, 1963; Johnson, 1991). It is widely used in the western half of the United States (Vogel and Moore, 1998) and in the saline soils of southern Australia (Smith, 1996). It has also been sown in low-rainfall

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environments and nonsaline soils of Australia (Cameron, 1959) and New Zealand (Douglas and Foote, 1994) for soil conservation purposes. It is one of the few improved species capable of growing in the poorly drained and alkaline soils of the Salado Region in Argentina (Mazzanti et al., 1992), which is one of the most important regions for beef cow-calf operations.

In the Salado region, tall wheatgrass is grazed by cattle, and in the vegetative stage it has acceptable nutritional value. However, tall wheatgrass can grow very rapidly in late spring, and once in the reproductive stage, it rapidly loses quality through reduced CP and increased NDF concentrations (Mazzanti et al., 1992). The uneven seasonal growth is often conserved in summer. The conserved forage is usually poor quality and is sometimes used as a sole diet when pasture growth is practically nil. Quality can be improved by harvesting earlier, but this requires skilled pasture management (Romera et al., 2005).

Reliable estimations of digestibility are needed to develop pasture management approaches that overcome the wide variation in tall wheatgrass quality. Nevertheless, there is still little information regarding the accuracy of digestibility estimation techniques for conserved tall wheatgrass. An experiment was undertaken to compare the precision and accuracy of 5 techniques used to estimate the in vivo digestibility of tall wheatgrass hay and haylage fed to cattle.

MATERIALS AND METHODS

Experimental Settings

The in vivo apparent digestibility of tall wheatgrass hay and haylage in ad libitum feeding conditions was determined during June and July of 2005 in an indoor experiment at the Argentinean National Institute of Agricultural Technology (INTA), Balcarce Experiment Station (37°45′ south; 58°18′ west).

A range of feed qualities were generated by growing pastures for different periods of time after defoliation. Six treatments were evaluated, consisting of 3 regrowth periods [short (66 d), medium (96 d), and long (162 d) regrowth] and 2 types of conservation (hay and haylage). Haylage was made on the same day of cutting, in rolls wrapped in white 25-μm polyethylene film. Hay rolls were made 4 d after each cutting, based on common practice. No precipitation was recorded between cutting and harvesting.

Forages were fed as a sole diet to 6 British steers, 20 mo of age and 337 ± 22 kg BW. The steers were housed in individual pens for three 14-d consecutive periods as replicates. Each steer consumed, sequentially, forages from the 3 regrowth periods but only one type of forage (i.e., either hay or haylage), following a crossover split-plot design. The first 9 d of each period enabled adaptation to the diet. In the following 5 d, measurements were taken of DMI, as the difference between offered and refused DM, and in vivo DM digestibility ($\mathbf{IV_{dig}}$) using the total collection method with feces bags and harnesses {IV_{dig} = [(intake − feces)/intake] × 100} on a DM basis (Schneider and Flatt, 1975). All the forages were fed twice daily in DM quantities that provided refusals of approximately 10%.

Sample Collection and Laboratory Analysis

Samples of approximately 3% of the feed offered, the feed refused, and the feces were collected daily during each of the 5-d measurement periods and were pooled by period for each animal. Therefore, 3 replicates of 2 conservation methods per 3 regrowth periods were analyzed. All the laboratory analyses were made in duplicate. Dry matter contents were determined in a forced-air oven (60°C) to a constant weight (approximately 48 h). Feed samples were milled to pass through a 1-mm Wiley screen and were analyzed to determine the concentrations of NDF, ADF, and lignin (ADL) by the filter bag technique, using an Ankom^{200} Fiber Analyzer (Ankom Technology Corporation, Fairport, NY). Crude protein was determined by total combustion in an ultrapure oxygen atmosphere (Horneck and Miller, 1998).

The technique of Tilley and Terry (1963) to determine in vitro apparent digestibility ($\mathbf{T\&T_{dig}}$) was used, including two 48-h digestion stages. In the first stage, 0.5 g of dried and milled forage sample, with 10 mL of rumen liquor (obtained from a fistulated cow fed alfalfa hay, corn grain, and sunflower pellets) and 40 mL of buffer solution, was anaerobically incubated in tubes at 38°C and pH between 6.7 and 6.9. In the second stage, 1 mL of 5% $HgCl_2$ and 2 mL of $2N$ Na_2CO_3 were added, the tubes were centrifuged, and 50 mL of pepsin solution was added to the residue, followed by incubation for 48 h at 38°C. Finally, the tubes were centrifuged and the residues were washed and dried to determine DM digestibility.

A Daisy^{II} Incubator (Ankom Technology Corporation) was used to determine in vitro true DM digestibility ($\mathbf{DAISY_{dig}}$). Dried samples (0.5 g) were weighed into filter bags and placed in a digestion jar that contained 1,600 mL of buffer solution and 400 mL of rumen fluid, and were incubated for 48 h. At the completion of the incubation, filter bags were rinsed and placed into the Ankom^{200} Fiber Analyzer (Ankom Technology Corporation) to determine NDF. Digestibility was determined as $DAISY_{dig}$ = {100 − [final weight − (bag tare weight × blank bag correction)]/(sample weight × DM) × 100} − 11.9, as recommended by Van Soest (1994).

In vitro DM degradability was determined using the in vitro gas-production technique ($\mathbf{GAS_{deg}}$; Villalba, 2001) at 3, 6, 12, 24, 48, and 72 h, and the 72-h determination was compared with IV_{dig}. Fermentation was carried out in 125-mL glass flasks sealed with rubber stoppers and aluminum retainers. The rumen fluid used was the same as described above. Approximately 50 mL of inoculum (containing 10 mL of rumen liquor, 8 mL of buffer solution, ammonium sulfate, and distilled water) was added to the flask and kept under CO_2 in a water bath at 39°C (Villa-

lba, 2001). Feed samples of 0.3 g DM were added to each flask, flushed with CO_2, and sealed. Two blank flasks were used to quantify gas generated by rumen liquor and its residue, and each sample was incubated in duplicate. The amount of gas produced was estimated indirectly by measuring the accumulated pressure in the headspace, using a hypodermic needle through the rubber stopper connected to an electronic manometer. The gas was released after each measurement. Finally, the GAS_{deg} was estimated by relating the volume of gas produced to that of samples of known degradability incubated in the same assay.

Two digestibility prediction equations based on fiber content of feeds were tested: a) Van Soest (1967; $\mathbf{VS_{dig}}$) {DMD (%) = [0.98 × (100 − NDF) + {147.3 − 78.9 $\log_{10}$[(ADL/ADF) × 100]} × (NDF/100)] − 12.9}, and b) Rohweder et al. (1978; $\mathbf{Ro_{dig}}$) [DMD (%) = 88.9 − (ADF × 0.779)].

Statistical Analysis

A split-plot design was used in the analysis, where conservation method constituted the main plot and the regrowth period was the subplot (Federer, 1955). The statistical model to compare the different feeds included the effects of period of regrowth, conservation method (hay or haylage), animal within conservation method, and the interaction between conservation method and period of regrowth.

The goodness-of-fit of all the techniques with IV_{dig} and the relationships among them were evaluated by simple linear regression analysis using the REG procedure (SAS Institute, 1999). The hypotheses of parallelism (equal slopes) and coincidence (equal slopes and intercepts) were also tested using dummy variables in the REG procedure of SAS.

The Pearson correlation coefficient, used to evaluate the strength of the association between observed and estimated data (in this case, with in vitro techniques), is one frequently used measure of precision (Lin, 1989; Iantcheva et al., 1999; Damiran et al., 2008). The concordance correlation coefficient ($\boldsymbol{\rho_c}$) proposed by Lin (1989) was also used. The coefficient ρ_c (scaled between 0 and 1) is a reproducibility index that evaluates the agreement between 2 sets of data by measuring the departure from the 45° line through the origin (the concordance line) in the observed versus predicted plot. The coefficient ρ_c integrates both precision (correlation coefficient, ρ) and accuracy (correction factor, $\mathbf{C_b}$) into a single indicator. Any departure from the concordance line would produce ρ_c lower than 1, even if ρ were 1. According to Lin (1989), C_b indicates how far the best-fit line deviates from the concordance line. Correlation, on the other hand, measures how far each observation deviates from the best-fit line, but fails to detect any lack of accuracy (i.e., a departure from the concordance line). A systematic divergence from the line of concordance corresponds to a bias in the estimations and can be characterized in terms of the slope (scale shift) and intercept (location shift) of the best-fit line (Pell et al., 2003). In the absence of biases, scale shift and location shift would take values of 1 and 0, respectively.

Table 1. Means (±SD) for the percentage of DM, NDF, ADF, ADL, CP, in vivo DM digestibility (IV_{dig}), and DMI (as a percentage of BW) of tall wheatgrass (*Thinopyrum ponticum*) hay and haylage after 3 regrowth periods [short (66 d), medium (96 d), and long (162 d) regrowth]

		Regrowth		
Forage	Variable (%)	Short	Medium	Long
Hay	DM	86.0 ± 0.6[ab]	84.2 ± 1.3[b]	86.9 ± 0.3[a]
	NDF	65.2 ± 0.3[c]	74.4 ± 0.9[a]	68.9 ± 1.7[b]
	ADF	42.4 ± 1.2[b]	48.1 ± 0.7[a]	46.9 ± 2.0[a]
	ADL	5.6 ± 0.4[a]	5.9 ± 0.5[a]	6.6 ± 0.5[a]
	CP	11.9 ± 1.2[a]	9.3 ± 0.7[b]	7.1 ± 0.5[c]
	IV_{dig}	50.4 ± 4.6[a]	53.2 ± 0.7[a]	42.2 ± 7.1[b]
	DMI	1.96 ± 0.48[a]	1.65 ± 0.43[b]	1.48 ± 0.28[c]
Haylage	DM	57.4 ± 2.8[a]	40.5 ± 1.3[b]	54.2 ± 1.4[a]
	NDF	61.3 ± 0.7[c]	65.3 ± 0.8[b]	72.0 ± 1.4[a]
	ADF	41.2 ± 0.6[c]	44.9 ± 0.1[b]	51.2 ± 0.1[a]
	ADL	5.1 ± 0.3[b]	5.2 ± 0.5[b]	8.0 ± 0.3[a]
	CP	13.7 ± 0.6[a]	9.5 ± 0.2[b]	6.9 ± 0.2[c]
	IV_{dig}	53.3 ± 2.9[a]	53.2 ± 6.0[a]	43.4 ± 4.3[b]
	DMI	2.13 ± 0.15[a]	1.88 ± 0.24[b]	1.57 ± 0.26[c]

[a–c]Different letters within rows indicate statistical differences ($P < 0.05$; Tukey's test).

RESULTS AND DISCUSSION

Chemical Composition and In Vivo Digestibility of Feeds

As expected, the NDF and CP fractions of forages were different ($P < 0.001$) between regrowth periods for hay and haylage (Table 1). For hay, the content of ADF ($P < 0.01$) was lowest in the short regrowth period, whereas the ADL content remained more or less constant ($P = 0.08$). In the case of haylage, there were differences ($P < 0.001$) in ADF content between all regrowth periods, and ADL content was lowest in the short and medium regrowth periods ($P < 0.001$), with both tending to increase with the duration of regrowth (Table 1).

There were no differences in IV_{dig} between hay and haylage. The IV_{dig} changed as the result of the increased regrowth periods ($P < 0.05$), generating a representative range for tall wheatgrass conserved forages, required to evaluate the digestibility estimation techniques. The long regrowth showed the lowest IV_{dig}

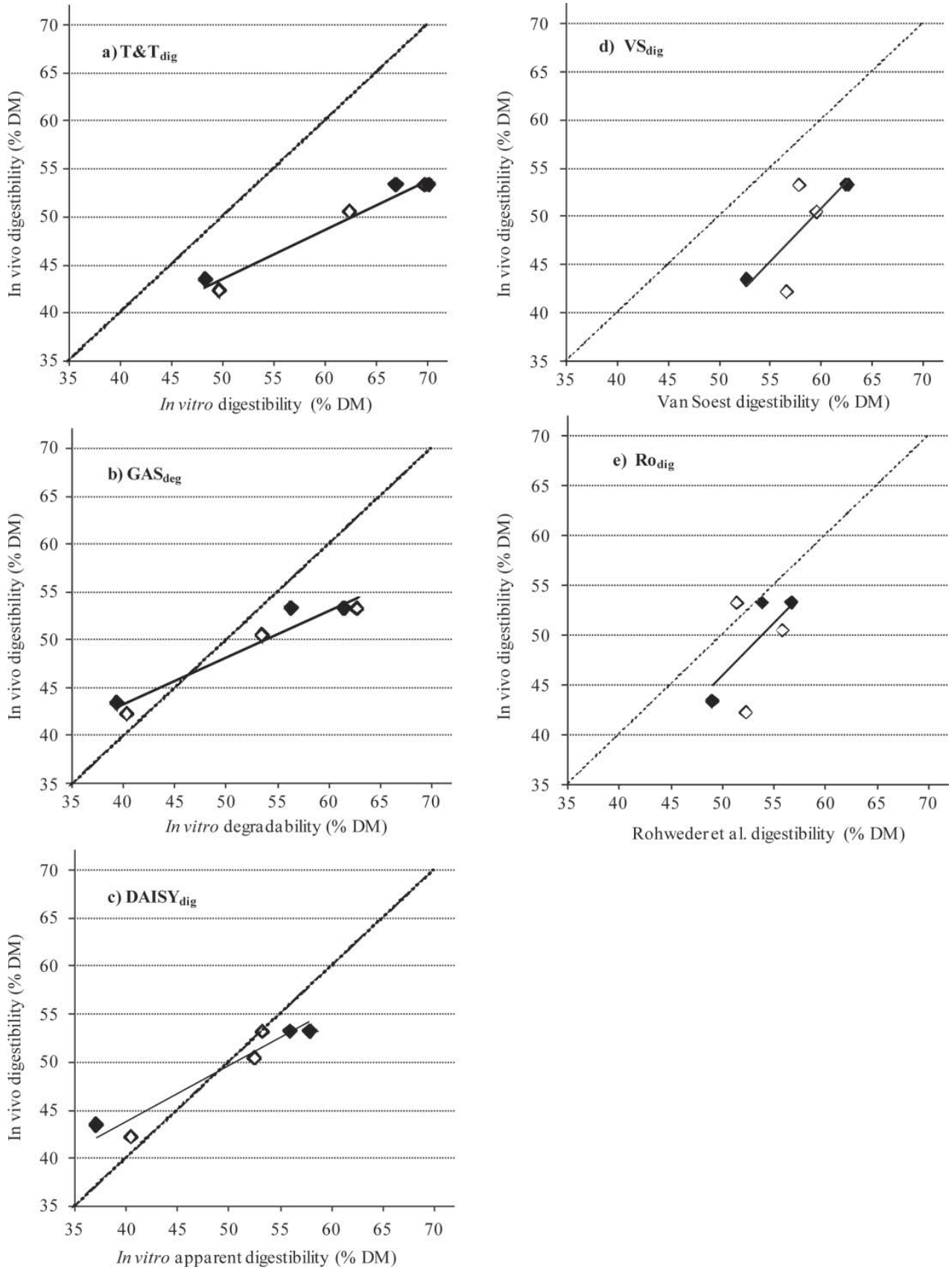

Figure 1. Relationship between in vivo digestibility and estimates: a) in vitro digestibility ($T\&T_{dig}$), b) in vitro degradability (GAS_{deg}) c) in vitro digestibility ($DAISY_{dig}$; Daisy^{II} Incubator, Ankom Technology Corporation, Fairport, NY), d) Van Soest (1967; VS_{dig}), and e) Rohweder et al. (1978; Ro_{dig}) for *Thinopyrum ponticum* hay (open) and haylage (solid). Regression (solid) and 1:1 or concordance (dotted) line.

Table 2. Regression analysis[1] between in vivo digestibility and in vitro digestibility estimates for tall wheatgrass (*Thinopyrum ponticum)* hay and haylage: in vitro digestibility ($T\&T_{dig}$); in vitro degradability (GAS_{deg}); in vitro digestibility ($DAISY_{dig}$)[2]; and the formulas by Van Soest (1967; VS_{dig}) and Rohweder et al. (1978; Ro_{dig})[2]

Estimate	Equation[3]	*P*-value	ρ	*P*-value, Ho:P	*P*-value, Ho:C	Location shift	Scale shift	ρ_c	C_b
$T\&T_{dig}$	y = 17.758 ± 2.77 + 0.515 ± 0.04x	0.0003	0.98	0.0001	<0.0001	−1.829	0.523	0.341	0.346
GAS_{deg}	y = 23.677 ± 3.25 + 0.489 ± 0.06x	0.001	0.97	0.0008	0.001	−0.464	0.504	0.718	0.740
$DAISY_{dig}$	y = 20.599 ± 4.01 + 0.578 ± 0.08x	0.002	0.96	0.0049	0.015	−0.050	0.600	0.850	0.881
VS_{dig}	y = −14.969 ± 23.89 + 1.095 ± 0.40x	0.054	0.80	0.797	0.006	−2.332	1.363	0.213	0.265
Ro_{dig}	y = −6.891 ± 38.57 + 1.055 ± 0.72x	0.218	0.59	—	—	−1.121	1.791	0.327	0.555

[1]ρ = Pearson correlation, Ho:P = parallelism, Ho:C = coincidence; location shift = bias from the intercept; scale shift = bias from the slope; ρ_c = concordance correlation coefficient; C_b = bias correction factor.

[2]Daisy^{II} Incubator (Ankom Technology Corporation, Fairport, NY).

[3]Parameter estimate ± SE.

(42.8 ± 5.27%), whereas short (51.8 ± 3.77%) and medium (53.2 ± 3.79) regrowths were similar (Table 1). Even though the NDF and ADF fractions were different between short and medium regrowths, their IV_{dig} were similar (Table 1), pointing to a weak relationship between fiber content and IV_{dig}.

Relationship Between In Vivo Digestibility and Estimates

Linear relationships were observed between IV_{dig} and $T\&T_{dig}$, GAS_{deg}, and $DAISY_{dig}$, with high correlations (Table 2). This observation is consistent with many literature reports. However, as discussed earlier, the correlation is a measure of precision only, and other goodness-of-fit indicators such as ρ_c and biases are required to assess accuracy. For example, the $T\&T_{dig}$ technique had the highest correlation, followed by GAS_{deg} and $DAISY_{dig}$, which would suggest that $T\&T_{dig}$ was the best predictor. However, $T\&T_{dig}$ exhibited the lowest C_b (i.e., the lowest accuracy), and because of this, its ρ_c was also very poor.

The $T\&T_{dig}$ technique overestimated IV_{dig}, in the whole range of observed values, by 24.2% on average, and the overestimation increased as IV_{dig} increased (Figure 1a). Biases have been reported before with low- and medium-quality feeds, with both overestimations (Damiran et al., 2008) and underestimations (De Boever et al., 1988; Arthington and Brown, 2005), but in general with good correlation with IV_{dig}.

In vitro gas production and in vitro DM degradability ($\mathbf{GAS_{deg}}$) have shown good correlations with IV_{dig} in the past (Khazaal et al., 1993; Gosselink et al., 2004; Kamalak et al., 2005). This study was no exception, but the regression analysis showed overestimation of IV_{dig} above 46.3% and underestimations below this value (Figure 1b). In the present study, there was a strong correlation between GAS_{deg} and IV_{dig} after 24 h of incubation (Figure 2), which is consistent with the report of Menke et al. (1979). Other studies have reported different results, with the highest correlation at 36 h (Van Soest, 1994), whereas Khazaal et al. (1993) found somewhat erratic results. Degradability at 72 h of incubation was used to estimate IV_{dig}, and it presented the greatest ρ_c, the lowest location shift, and the C_b nearest to unity (data not shown), compared with the other incubation times.

A similar type of bias was observed with $DAISY_{dig}$, with overestimation above 48.8% and underestimation below this value (Figure 1c). Damiran et al. (2008) found a similar trend

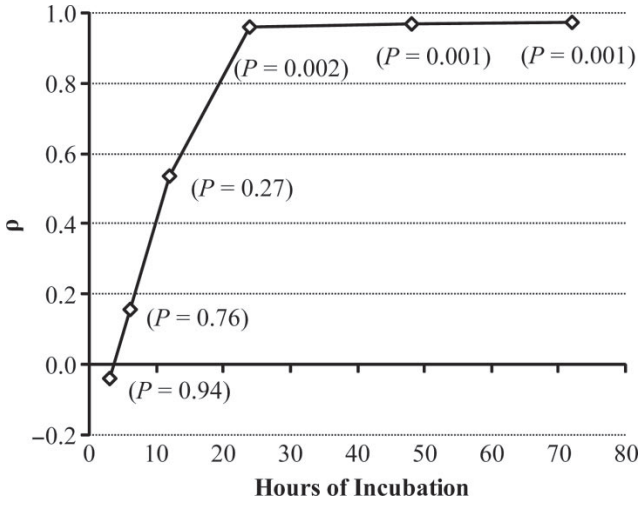

Figure 2. Relationship between Pearson correlation (ρ) of in vitro degradability by the gas production technique and in vivo digestibility in 6 h of incubation for 3 regrowths of *Thinopyrum ponticum* hay and haylage.

for $DAISY_{dig}$ when analyzing grass hay and straw. With grass hay, these authors found that $DAISY_{dig}$ overestimated IV_{dig} (0.71 vs. 0.62, respectively) but that the opposite occurred with straw (0.38 vs. 0.50, respectively).

The parallelism hypothesis between observed and predicted values was discarded for the 3 in vitro techniques; therefore, no technique showed outstanding values of ρ_c (Table 2). This means that all techniques displayed prediction biases (Table 2). Nevertheless, it is clear that the high correlation coefficients indicate, at least for this type of feed, that it would be feasible to calibrate these techniques to correct such biases.

Determining the causes of the biases (described above) was beyond the scope of this study. The biases associated with in vitro assays are a consequence of "simplification" of the digestion processes. Contributing variables include sample preparation (e.g., drying and grinding vs. freshly chewed feed; Chaves et al., 2006), prolonged digestion times, and the use of few enzymes to degrade complex structural carbohydrates and proteins. For example, the GAS_{deg} assay estimates only digestion in the rumen, without considering postruminal activity. Although buffers are used, pH changes during in vitro incubation, and the absence of nitrogen recycling could limit microbial growth when poor-quality feeds are digested. Conversely, in vivo digestion is affected by feed intake and passage rate through the rumen (Gosselink et al., 2004), but responses are inconsistent (Table 1) and vary between individuals. In vitro techniques will always be an approximation of true digestion, but they should demonstrate relativity commensurate with animal measurements.

The estimates from VS_{dig} achieved a rather low correlation with IV_{dig}, and its ρ_c and C_b (Table 2, Figure 1d) were lower than those obtained with the in vitro techniques; therefore, it was less precise and less accurate. The lower determination coefficient observed for VS_{dig} was due to the lack of correlation of IV_{dig} with NDF and ADF ($P = 0.48$ and 0.22, respectively), on which this equation relied. The correlation observed between the ADL fraction and IV_{dig} justified the moderate precision of VS_{dig}. Nevertheless, VS_{dig} presented large correlations with the in vitro techniques (Table 3).

The Ro_{dig} equation was inadequate in predicting IV_{dig} for this type of feed ($P = 0.21$) (Table 2, Figure 1e). According to Abrams (1988), after evaluating 60 forages, the low relationship between digestibility and ADF explains the low precision of this equation. Consistent with Abrams (1988), there was no correlation between IV_{dig} and ADF content ($P = 0.22$) in the present study. Khazaal et al. (1993) and Moore and Coleman (2001) reported correlations between IV_{dig} and ADF ranging from −0.39 to −0.93. Therefore, this methodology is not sufficiently accurate to predict IV_{dig} for tall wheatgrass reserves.

In summary, the 3 in vitro techniques achieved high correlations with IV_{dig}, but $DAISY_{dig}$ showed the greatest ρ_c, the lowest location shift from the line of concordance, and the C_b nearest to unity (Table 2). The $T\&T_{dig}$ technique, on the contrary, showed a very low ρ_c because of a very high location shift.

Relationships Among Digestibility Estimation Techniques

All in vitro estimations presented correlations among each other greater than 90% (Table 3). The $T\&T_{dig}$ technique produced the highest estimations ($P < 0.05$), followed by GAS_{deg} and $DAISY_{dig}$.

The $DAISY_{dig}$ technique was parallel to $T\&T_{dig}$ ($P = 0.51$), but y-intercepts differed and therefore were not coincident because $T\&T_{dig}$ values were considerably higher than those for $DAISY_{dig}$ (see Figure 1). This is similar to the results reported by Holden (1999) and Vogel et al. (1999). Damiran et al. (2008) mentioned that these techniques ranked the samples in a similar order, but in that case, $DAISY_{dig}$ estimations were higher than those of $T\&T_{dig}$.

In addition, the pair $T\&T_{dig}$ and GAS_{deg} was highly correlated and parallel ($P = 0.74$), but again not coincident, with $T\&T_{dig}$ being notably higher than GAS_{deg}. This is in agreement with Cone et al. (1999), although these authors found lower values for $T\&T_{dig}$ than for GAS_{deg}.

An even greater agreement was observed between GAS_{deg} and $DAISY_{dig}$, being highly correlated and statistically coincident ($P = 0.22$). Therefore, there is no practical distinction between the techniques.

The VS_{dig} showed high correlations with the 2 more accurate in vitro techniques ($T\&T_{dig}$ and $DAISY_{dig}$). Because of this, VS_{dig} could be

Table 3. Pearson correlation coefficients (under the diagonal) and *P*-values (above the diagonal) between in vitro digestibility ($T\&T_{dig}$), in vitro degradability (GAS_{deg}), in vitro digestibility ($DAISY_{dig}$),[1] and the formulas by Van Soest (1967; VS_{dig}) and Rohweder et al. (1978; Ro_{dig}) for tall wheatgrass (*Thinopyrum ponticum*) hay and haylage after 3 regrowth periods [short (66 d), medium (96 d), and long (162 d) regrowth]

Estimate	$T\&T_{dig}$	GAS_{deg}	$DAISY_{dig}$	VS_{dig}	Ro_{dig}
$T\&T_{dig}$	—	<0.001	<0.001	<0.05	0.25
GAS_{deg}	0.99	—	<0.001	0.08	0.34
$DAISY_{dig}$	0.96	0.92	—	<0.01	0.07
VS_{dig}	0.82	0.76	0.92	—	0.03
Ro_{dig}	0.55	0.48	0.77	0.86	—

[1]Daisy[II] Incubator (Ankom Technology Corporation, Fairport, NY).

regarded as an approximation for tall wheatgrass reserves when the other techniques are not available.

Practical Considerations

Apart from the fact that $T\&T_{dig}$ was less accurate than the other 2 in vitro techniques, it is also a protracted (96 h of digestion) and time-consuming technique. The Daisy system displays good agreement with (but less bias than) $T\&T_{dig}$, as confirmed in the present study, but it is faster and easier to run because all the analyses are done in the same digestion vessel (Holden, 1999; Vogel et al., 1999; Wilman and Adesogan, 2000).

In some cases, not only digestibility but also digestion rates are required. In that sense, unless lengthy and labor-intensive studies are used, neither the $T\&T_{dig}$ nor the $DAISY_{dig}$ provides such extra information (Getachew et al., 1998). Two roughages may show the same digestibility at 48 h, but degradation rates may differ (Blümmel and Becker, 1997). These techniques are known as end-point measurements, because they give only one final measurement and the residue determinations destroy the sample. The gas-production techniques, on the other hand, give information about the extent of degradation and the degradation kinetics of feeds (Theodorou et al., 1994; Williams, 2000).

In summary, $T\&T_{dig}$ overpredicted all qualities and is time consuming. The $DAISY_{dig}$ technique is fast and accurate, so it would be the most applicable. The GAS_{deg} is slightly less accurate than $DAISY_{dig}$, but provides extra information on digestion rates. Therefore, the recommended techniques are $DAISY_{dig}$ or GAS_{deg}, depending on requirements. However, all in vitro techniques showed biases, highlighting certain limitations for conserved forages.

IMPLICATIONS

In the present study, digestibility estimations for wheatgrass reserves, by the most common techniques, showed high correlation with in vivo digestibility, but also noticeable biases. This is the general situation observed for roughages in the literature. Unfortunately, the nature and size of the biases are not consistent between studies, which precludes the use of simple correction formulas. In addition, the question remains about the repeatability of the biases within the same feed. Nevertheless, digestibility estimation techniques do provide an indication of the quality of forages. They can still be very useful, provided the user is aware that, although the estimation techniques provided are well correlated with in vivo digestibility, they do not necessarily predict it accurately.

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