Standardizing Digestibility Results Across Labs: A Possible Approach

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Key Points

- Present approaches for measuring digestibility of feed fractions (extents of digestion at fixed time points or rates), particularly for fermentation assays, generate values that vary within and among labs, and among methods. This variation does not allow for great precision in specifying the digestibility values.
- Accuracy of predictions by energy calculations and models may be reduced by the variability of digestibility values, or if the equations are sensitive to the input but were not calibrated to the range of values produced by a method or lab.
- Labs are frequently able to rank feeds similarly, although the values they generate may differ.
- It may be possible to standardize digestibility results if labs included feedstuff standards representing a range of digestibilities for a feed fraction in each fermentation run. Results of the standard feeds could be used to rank feeds as high, medium, or low. These qualitative grades could then be assigned a numeric digestibility value that is consistent with the range of values utilized in equations/models.
- Use of a ranking system based on common fermentation standards could increase the coherence and applicability of digestibility values by reducing the effects of the variability inherent in biologically-based assays.

One of the challenges of working with both rate of digestion and extent of digestibility at a fixed time point is that the values vary within and across laboratories, and by method. For example, when the 30 hour extent of neutral detergent fiber (NDF) digestion of a control corn silage sample was measured in 1 lab over 7 months (n = 659) the mean value was 53.6%, with a standard deviation of 1.8, and a measured range of 41.6% to 61.8%. A range of $\pm$ 3 standard deviations, which represents approximately 99.7% of the values is 10.8 percentage units, or 48.2% to 59.0% digestibility. In comparison, the range of 30 hour NDF digestibilities reported for all corn silage samples analyzed in that lab in that time period was 28 to 80% (n = 5838).). The variability in the measurements of the single control sample represented 20% of the total variation reported for all forages of this type. Similar or greater variation in digestibility results within or across labs is not uncommon. The precision of the measured values dictates what precision is reasonable to demand for use of these values in models or equations (Hall and Rymph, 2005). With our present methods, our ability to measure the digestion rates or extents of digestion at fixed time points with fine precision (+/- 1 percentage unit) that is repeatable over time and within and among labs is not a reality.

The impact of the variability in measured digestion values depends on how they will be used. Currently, these values are used by field nutritionists to compare feedstuffs and qualitatively (e.g., the feed fraction is more or less digestible than the average) adjust feed energy values, to estimate energy content of feeds through equations (NRC, 2001), and in nutritional models. The predicament with using these values in energy equations or models is that the lab generated values may or may not align with the “true value” or with the range to which a model was

calibrated. The magnitude of the impact depends on the sensitivity of the models/equations to the input. The impact of a single digestibility value on a model is a function of the magnitude of the digestibility value for a feed, the proportion of that feed that is represented by that fraction (e.g. NDF) and the proportion of the ration that is that feed. The effect of variation for each feedstuff may also be compounded by the number of feeds in the ration. However, the impact may be greater or lesser depending on the relationship of the digestibility value to other factors in the model (Hall, 2004).

A potential solution to making imprecise digestion values more useful is to use fermentation standards and apply a ranking system. This approach could help to make the values more coherent despite the variation in the measured values and thus make them more useful in various applications. This approach relies on the ability of laboratories to consistently rank samples in the same order. Other researchers have recommended the inclusion of standard samples in in vitro fermentations as "a prerequisite for leveling out the varying activities of rumen juice and establishing an accurate correlation with in vivo digestibility values" (Gruber et al., 1998) (also Knipfel, 1976). The qualitative ranking of samples for NDF digestibility was suggested in 2002 by Mertens (presentation to NIRS Consortium meeting; see Powerpoint presentation on this website). These rankings could potentially be converted to categorical values for use in models where numeric inputs are required. Grouping of results into ranges places more reasonable expectations of precision and improves repeatability of results without impairing the utility of the results.

Proposed Method:

- The forage/feed standard samples used should roughly bracket the high and low ends of the expected range of digestibility or rate values for the feed and feed fraction evaluated.
- In each fermentation run, two standard samples would be included (in duplicate) that correspond to the forages/feeds to be evaluated (for alfalfa haylage, use alfalfa haylage standards, for corn silage use corn silage standards, etc.).
- Use the digestibility values for the standards to divide the range of values into thirds, which would correspond to high, medium, and low digestibility or rate (could split this into just high and low values depending on variability of assay, range of values, etc.). Monitoring rolling mean digestibility values of the standards over time could assist with determination of errant values of replicate standard samples within fermentation runs. However, great differences between replicates within run may speak to problems with the analytical method and its execution, rather than to values to be excluded.
- The labs could report the values of the standards and individual feed and the feed's rank.
- For use in modeling applications, the rank would be used to select a rate or digestibility value corresponding to the values that parse into high, medium, or low in the program. The modelers will have to prescribe the values needed for their model based on the ranking system; that may be affected by the range of digestibility values on which the model was developed/calibrated.

In the example below (fabricated, not from real labs), two labs have single time point NDF digestibilities for a type of feed. To calculate the range of numbers that describe different ranks: (High standard - low standard + 1)/3 = 1/3 of the range. (The "1" is added to encompass the entire range of values.) The lower bound of the high range = the high standard - (1/3 of the

range) + 1. The upper bound of the low range = the low standard + (1/3 of the range) - 1. The medium range is between the upper bound of the low range, and lower bound of the high range.

Example: Values for a fixed time point fermentation for NDF digestibility (% of NDF digested).

Sample	Lab 1	Lab 1 Standard Ranking	Feed Rank	Lab 2	Lab 2 Standard Ranking	Feed Rank
High Standard	54	High: ≥47		48	High: ≥ 43	
Feed A	41	Medium	Medium	37	Medium	Medium
Feed B	52	<47, >39	High	47	<43, >35	High
Feed C	30		Low	28		Low
Low Standard	32	Low: ≤ 39		30	Low: ≤ 35	

In this example, if the labs rank samples similarly, although the numeric values are not equivalent, the rankings based on standards are. There is potential for dissimilar rankings for samples that fall close to the border of a rank.

For this system to work, the same standards would be used across labs and fermentations in a given year. It is likely that new standards would have to be developed and distributed every one or two years. It is possible that an approach could be developed in which fewer standard samples could be used to assess digestibility rank.

Literature Cited

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