Starch Digestibility Metrics – Overhyped or Overfit?

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Many metrics for quantifying aspects of starch digestibility (**SD**) are available through commercial laboratories, but a robust system for predicting SD across feed types is lacking. Today's metrics are useful for answering specific questions about specific feed types, but an ideal diet formulation system needs the ability to quantify differences across feed types to enable reliable feed purchasing, procurement, and formulation decisions. The most utilized methods today, including near infrared spectroscopy, in vitro, and in situ analyses have significant shortcomings, including in their inability to quantify the impact of particle size on SD. In vitro gas production analysis provides unique opportunities to overcome these shortcomings, but adaptation requires further validation of the method and changes in the handoffs between lab measurements and diet formulation programs.

Introduction

A plethora of SD metrics are available through commercial labs, including corn silage processing scores (**CSPS**), grain particle size, prolamin, amylose/amylopectin, vitreousness, ammonia, soluble protein, in vitro and in situ analysis at various time points, starch gelatinization scores, and fecal starch. Still, one of the most important questions about SD remains difficult to answer: how to account for it in diet formulation. While the metrics available today are useful for answering specific questions about specific feed types, they fall short of creating a robust system that can be used to make quantitative comparisons across feed types. This paper will attempt to provide clarity around practical uses of today's metrics, where they fall short of ideal, and hurdles that need to be overcome to create better systems in the future.

CSPS, grain particle size, prolamin, amylose/amylopectin, vitreousness, and starch gelatinization scores can be used to answer specific questions within a narrow subset of feed types. They have been covered in other papers and will not be discussed in depth here. Feed Grain 2.0, developed at the University of Wisconsin, integrates particle size with ammonia or prolamin to make predictions about digestibility and provides inputs for diet formulation, but it is only designed for use with corn grain, snaplage, and earlage. On the other end of the cow, fecal starch is a reliable metric for detecting poor SD in a diet. Measured with or without an internal marker (lignin, uNDFom120, uNDFom240, ect.), it can also be used to calculate the digestibility of starch in the diet (Fredine et al., 2014). However, fecal starch is only an in indication of SD in the total diet after it has already been fed. An ideal system for diet formulation needs to predict SD of individual feeds before they are fed to enable purchasing and management decisions.

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NIRS is Not Enough

Near infrared reflective spectroscopy (**NIRS**) has allowed a rapid expansion in the number of parameters that are used as inputs for diet formulation. A full set of formulation inputs for common programs today would cost more than $300/sample and take more than 3 weeks in a laboratory if performed by chemistry but are available within 1 day for less than $30 by NIRS. However, SD is fundamentally different than other formulation parameters because it is sensitive to particle size. Protein, NDF, and mineral concentrations do not change when a sample is ground to a fine powder, but SD is directly impacted by particle size (Fernandes et al., 2018). In every commercial laboratory the author is aware of, NIRS analysis is performed on a sample ground to pass a 1mm screen. This type of NIRS analysis cannot contain information about particle size. Even if samples were scanned without grinding, most NIRS instruments are designed to scan the sample from the bottom up, which results in an over-representation of fine versus coarse particles. Unlike most formulation parameters being used today, NIRS alone is not able to measure the key variables in SD.

Bags and Washout – a Feature or a Bug?

All in situ analyses, and some in vitro methods, rely on placing samples in a filter bag and then placing that bag in rumen fluid, either through a rumen cannula or in an in vitro vessel. These types of analyses reduce labor costs, may increase the repeatability of a method, and in the case of in situ analyses, reduce the overhead costs of operating a laboratory. If bags are sized large enough, these methods also eliminate the need to grind samples prior to analysis (Nocek, 1987). However, any bag method requires assumptions to be made about small particles that escape the bag prior to incubation. Traditionally, this issue was thought of as a bug in the analytical methods with the undigested particles escaping the bag referred to as "washout", which can be measured by washing the bags in water prior to incubation. More recently, this issue has been recast as a feature, with the undigested particles escaping the bag referred to as "small particle starch" or "soluble starch". Semantics aside, it is reasonable to assume that small particles of starch are more digestible than large particles and quantifying their presence may be useful.

It is necessary to translate these metrics into input parameters for the purpose of diet formulation, and without directly analyzing the digestibility of washout particles, it is necessary to make assumptions about their digestibility. Several approaches have been used in research for assigning digestibility of this washout fraction, including assuming instantaneous digestion (Orskov and McDonald, 1970), assuming a digestibility greater than the remaining sample (van Duinkerken, 2011; Volden, 2011), or ignoring the washout fraction and assuming all particles digest at the same rate (Johnson et al., 2003).

In work performed at Dairyland Laboratories Inc., Schlau et al. (2020a) selected corn grain samples for a range in maturity (pre-corn silage maturity through dry grain) and extent of fermentation (16.7 to 54.1% soluble protein), separated washout and retained sample using filter bags, and measured their digestion kinetics through gas production analysis. To our surprise, fermentation rate (kf, %/hr) was not statistically different between washout and retained particles. The big difference between washout and retained particles was that fermentation of washout material started almost immediately (lag = 0.65 hr), while retained particles had a long delay before fermentation began (lag = 4.88 hr). In other words, small and large particles fermented at approximately the

same rate, but large particles took much longer to get started.

While it is commercially feasible to measure the amount of small and large particle starch, their respective fermentation rates, and their respective lag times, incorporating this information into formulation programs would require changes in the assumptions these programs make about lag. Today, all commercial programs the author is aware of utilize lag as an input to calculating the fractional rate of digestion (**kd**) and make the implicit assumption that lag is a characteristic of the lab. Schlau et al. (2020a) suggests that lag is a characteristic of the feed which would need to be subtracted from the rumen retention time before calculating digestion with a kd/(kd+kp) approach. If starch has a mean retention time of 7 to 9 hours, certainly it is important to account for starch that does not begin fermentation until hour 4.

Starch is Not Fiber

Fiber analysis and systems for predicting digestibility have evolved through an iterative process of quantifying a portion with a known digestibility and gradually narrowing the portion with a variable or unknown digestibility. For example, Goering and Van Soest (1970) created the NDF procedure to separate nutrients with a relatively small range in digestibility (protein, fat, ash, sugar, ect.) from nutrients with a wide range in digestibility (hemicellulose, cellulose, and lignin). Later, the indigestible fraction of NDF was defined first by lignin (Traxler et al, 1998), then by undigested NDF (**uNDF**) measured at 240 hours (Palmonari et al., 2017). Most recently, there have been attempts to separate fast, slow, and indigestible fiber using multiple uNDF time points (Raffrenato et al., 2019).

Starch is not fiber. Besides the previously mentioned challenge that NIRS is not able to interpret the impact of particle size on SD, we have not been able to measure an appreciable amount of indigestible starch in our laboratories with more than 95% in vitro starch digestibility (**IVSD**) at 24 hours for all samples (Mertens et al., 2017) and 100% IVSD at 72 for all samples in an unpublished study of dry corn grain. While there are references to indigestible starch in other industries, it is important to note that the AOAC method for dietary starch developed by Dr. Mary Beth Hall intentionally eliminated components of other starch methods that are indigestible to livestock (Hall, 2019). We have also found that SD time points are highly correlated and add little to our ability to calculate starch kd's (Mertens et al., 2017).

Hybrid Selection and Diet Formulation Require Different Metrics

Much of the research around SD metrics in our laboratories is funded by companies trying to evaluate and quantify SD differences among hybrids.When we introduced a commercial IVSD7 analysis in 2009, the selection of 7 hours as a time point had nothing to do with diet formulation but was chosen strictly because it was the time that maximized the observable difference between known flinty and floury corn samples (unpublished). However, while tools for hybrid selection only need to differentiate samples, tools for diet formulation need to account for additional management variables that can occur at the farm, including degree of processing (particle size) and the impact of ensiling on SD over time.

As an industry, we could serve both needs better by recognizing that, while they are related, these are separate questions that require separate laboratory measurements and indexes. For example, energy related metrics like milk per

ton are often used to compare hybrids because they allow starch and fiber concentration, as well as digestibility, to be built into a single metric. However because SD changes with kernel processing and ensiling, comparing milk per ton from samples with different kernel processing or extent of fermentation does nothing to improve hybrid selection.While laboratory methods can certainly be improved, today's methods are sufficient for detecting differences in SD of hybrids if stage of maturity and particle size are controlled (Schlau et al., 2020b). However, metrics intended for diet formulation must take the opposite approach. They need to incorporate variation due to particle size and stage of maturity instead of isolating them.

What is Practical Today?

IVSD measured by NIRS is a practical tool for managing variation in SD due to extent of fermentation.With the reference method run on 4 mm Wiley ground samples, single time point kds with a fixed lag timPe result in digestibility predictions with major feed types ranked appropriately (Figure 1) and within similar ranges as published research (Table 1). However, this does not measure all variation that matters to the cow (Coons et al., 2019) and may not be practical for alternative starch sources, like steam flaked corn, small grains, and pure corn starch due to their unique physical characteristics.

Alternatively, and perhaps just as effectively, Feed Grain 2.0 can be used for formulation inputs on corn grain, snaplage, and earlage, and expanded to provide inputs for corn silage using CSPS and ammonia or soluble protein.That still leaves steam flaked corn, extruded corn, and other starch containing feeds to be defined.

Where Can We Go in the Future?

Gas production analysis provides unique opportunities because it is sensitive to carbohydrate digestion and provides a virtually unlimited number of time points that make it mathematically feasible to define lag and fermentation rate for starch as well as other carbohydrate pools. It is also possible to scale up in vitro gas systems to analyze samples without prior drying and grinding. This may be useful in avoiding the confounding impacts of kernel vitreousness with grinding. Finally, gas measurements can be made in real time and do not require additional filtration and chemistry, which means turn-around times of 2 to 3 days are practical with lower marginal cost than even single time point IVSD or NDFD measurements.

However, gas production analysis has credible skeptics with credible reasoning. First, when gas production is measured, all we can say for certain is that a carbohydrate was digested at a given time. We may not be able say with certainty whether the gas was produced from sugar, starch, or NDF fermentation. Second, the relationship between the volume of gas produced and the mass of carbohydrate digested is not fixed. Fermentations that produce acetate produce more gas than fermentations that produce propionate (Van Soest, 1994). Third, there may be interactions between starch, fiber, and feed additives that change fermentation pathways and therefore the amount of gas that is produced by a feed sample.

To examine these concerns, we have run several internal trials aimed at proving whether these concerns are valid and developing a more robust system for quantifying SD for diet formulation. In the first trial (Knapp et al., 2021), we developed a mathematical approach to derive fermentation curves for sugar, starch, and fiber pools from the gas production curve of

an entire sample. This approach was first tested in a Monte Carlo simulation to evaluate whether it would work over the expected range of starch and fiber digestibilities for corn grain and corn silage. This work confirmed the mathematical approach was robust over the range of expected nutrient values and could determine lag and kd for each carbohydrate pool, even with the inherent analytical variation of an in vitro gas production system.

In a follow up trial, we tested a set of corn silage samples with a range of starch and fiber digestibilities through the gas system and mathematical model, concluding that the approach was able to predict kds with reasonable accuracy and provided a wider range of values than in vitro starch and NDF digestibilities, suggesting that it may be a more sensitive measure (Knapp et al., 2021). The improved sensitivity of this approach is due, in part, to the ability to predict lag times for individual feed samples, rather than assuming a universal value for all samples at an individual laboratory.

In a third trial, designed to determine whether interactions between starch and NDF would confound lag and kd predictions, composite samples were made with varying proportions of corn silage, dry corn, and high moisture ear corn. The 5-sample set contained a 100% grain sample, a 100% corn silage sample, and 3 samples with varying grain:corn silage. A model that included pools for the grain starch, silage starch, and silage NDF was used. We were able to statistically fit a single set of parameters (kds and lags for each pool) to all 5 samples in the set, demonstrating that the model is robust and that the gas production from both starch pools and the NDF pool were fully additive. A fourth, and final trial so far, was run by repeating this approach with corn grain samples and isolated NDF from the corn silage samples. The gas production from the grain starch and isolated NDF were again found to be fully additive (Schlau et al., 2021). This suggests that the stoichiometric difference in gas production from acetate or butyrate vs propionate may not be detectable due to interconversion of VFA in microbial metabolism and the presence of CO_2 utilizing pathways in propionate and methane formation. As an extension of this work, we are currently evaluating the effects of particle size and drying method on in vitro gas production.

Summary

In conclusion, today's commercially available metrics for SD are useful for a narrow set of questions and feed types, but the information is not always transferable/translatable to diet formulation. Also, there are several factors in in vivo digestion, for example rumen retention time and passage rates, which cannot be predicted from a feed analysis. New methods that can predict and quantify SD across feed types are needed to optimize ingredient purchasing, management, and formulation decisions. Gas production methods provide unique opportunities due to their ability to avoid issues with washout, to incorporate the impact of particle size on digestion, and to measure variations in lag that are a characteristic of the sample. However, to take advantage of advances in laboratory methods, changes may be needed to the handoffs between lab measurements and diet formulation programs.

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Table 1. Ferraretto et al. (2013) 202 trails - ruminal starch digestibilities from 24 to 80% and total tract starch digestibilities from 86 to 98%[1]. Huntington (1997) slightly higher ranges for ruminal (50 to 94%) and total tract (87% to 99%) starch digestibilities for processed grains[2]. SD = Starch digestibility, IVSD = in vitro SD, and MIR – mid-infrared reflectance.

IVSD % of Starch	MIR kd™ (%/hour)	Ruminal SD (%)	Ruminal SD (%)	Total Tract SD (%)	Total Tract SD (%)
25	4.8	37.5		89.4	
35	7.2	47.3	24[1], 50[2]	91.0	85[2], 87[1]
45	10.0	55.5		92.4	
55	13.3	62.5		93.6	
65	17.5	68.6		94.7	
75	23.1	74.3		95.6	
85	31.6	79.8		96.6	
95	49.9	86.2	80[1], 94[2]	98.4	98[2], 99[1]
99	76.8	90.6		98.4	

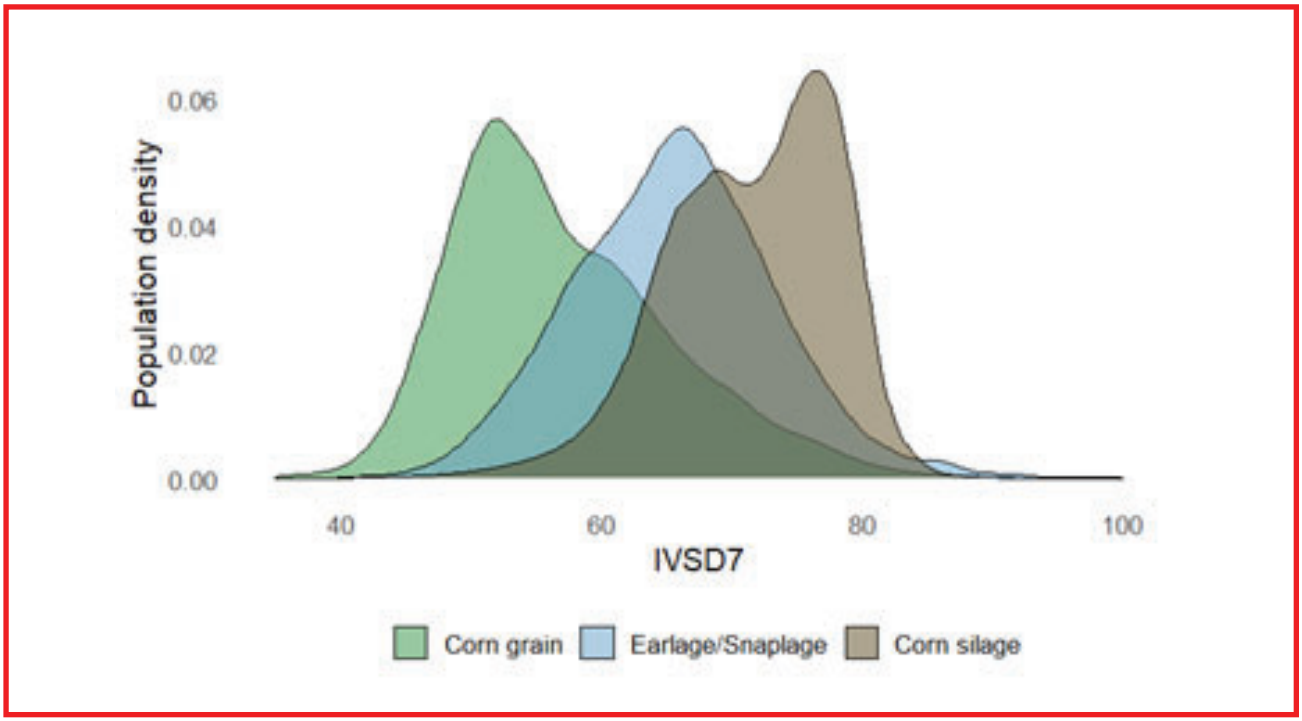

Figure 1. Distribution of in vitro starch digestibility (IVSD) across more than 200,000 samples analyzed by NIR at Dairyland Laboratories, Inc in 2020.