Practical Steps to Improve Diet Digestibility

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Introduction

NASEM (2021) took a large step forward predicting total tract digestibility of nutrients in a summative equation to estimate digestible energy. Starch and fatty acid **(FA)** digestibility have important implications on total diet digestibility, but they also (especially starch) can depress neutral detergent fiber **(NDF)** digestibility **(NDFD)**. Although we measure NDF, acid detergent fiber **(ADF)**, and acid detergent lignin **(ADL)** in commercial labs, the detergent system was designed to estimate hemicellulose **(NDF – ADF)** and cellulose **(ADF – ADL)** concentrations of forages, which would help advance our knowledge if we did this more often in our studies. Hemicellulose is very diverse in chemical constituents, and cellulose **(ADF – ADL)** is very different in its crystallinity and therefore digestibility (Firkins et al., 2025). Besides different forages, differences in maturity and environmental factors also influence chemical components in fiber. For example, plant breeding to decrease lignin can result in plant adaptation that increases phenolic cross-linking to hemicelluloses. On a smaller scale, microbes adapt to changing availabilities of those substrates over time after colonization. The net result is a heterogeneous mix of substrates and microbes that increases variation among animals fed different diets and even animals fed the same diets. In this paper, I will discuss some aspects that appear to increase or decrease NDFD.

Soluble fiber can be measured separately but is not recovered by the NDF assay and often is not measured in the field. Starch is now much more accurately measured than a couple of decades ago, but starch availability in the rumen is difficult to assess and can interact with expectations for NDFD, particularly in the rumen versus the large intestine. Sugars also can be measured separately from starch. However, if you include oligosaccharides as sugars, they vary among sources and can include oligosaccharides, not just simple sugars. NASEM (2021) explained why FA should be analyzed and ether extract basically buried in the same graveyard as crude fiber. In fact, if we assume only some FA (not other components of ether extract) can improve NDFD, then we should be exploring the individual FA in the total diet.

When modeling ruminal digestibility, our databases have gaps that are related to imbalanced meta-data. Both NDF and starch digestibilities in the rumen were plagued with such issues in efforts by NASEM (2021) committee members, although greater datasets allowed total tract digestibility of nutrients to be mined for the summative equation described previously. Because of imbalanced combinations

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of feed ingredients across studies, we must rely on concepts derived in individual studies. Alfalfa has been studied compared with orchardgrass to show how fragility affects NDFD and dry matter intake **(DMI)**, but that is generally outside of my current scope. Moreover, any such measurements of fragility are not consistently distributed across a variety of forages or different forage types such that ADF:NDF (a proxy for different forages) is merged with differences in ADF:NDF within forage type. Hence, undegraded NDF **(uNDF)** is widely assessed in the field, but this assay is not routinely measured in peer-reviewed research and even then, often is only compared to in vitro assessments of NDFD or lignin analysis. Therefore, uNDF discussions will be outside of my scope. Finally, we know how grain processing or vitreousness affects ruminal starch digestibility, but particle size is not routinely reported for grain across studies and is not on a DM basis for TMR in which wet forages do not distribute across particle size fractions. I will not discuss these topics, either, because they have been covered by previous authors.

The current objectives are to discuss updates and gaps for digestibility of different nutrient fractions with respect to gains from NASEM (2021) and cover aspects related to FA and sugars that can interact with NDFD (Firkins et al., 2025). When these topics overlap with potential gains in microbial protein production in the rumen, I will provide my perspective.

Starch and NDF Digestibilities in the Rumen and Total Tract

Many authors (including me) have discussed starch digestibility many times with respect to different sources of grain, vitreousness, and processing, and a good base on this topic is available through DAIREXNET (https://dairy-cattle.extension.org/starch-digestibility-of-corn-silage-and-grain/#Starch_Digestibility_of_Processed_Corn_Grain). NASEM (2021) has categorized starch digestibility coefficients as derived by multiple sources (Table 1). The default was 91%, whereas flaked corn and barley were classified as 94% but coarsely rolled corn (> 3500 µm) as only 77%. Increasing dryness of corn silage decreased starch digestibility from 91 to 85%. However, these are averages and probably extend lower on farms. In fact, dry corn silage was a major problem in the NASEM data that interfered with efforts to categorize rumen starch digestibility. For example, in a few studies, replacing ground corn with rolled barley increased ruminal starch digestibility, but these studies had low starch digestibility resulting from very high corn silage. Clearly, a key takeaway is to harvest corn silage at proper maturity and to consider inoculants and kernel processing (Ferraretto et al., 2018). Adding corn grain to overcome poor starch digestibility of corn silage introduces more variability, particularly as the silage stews and the kernels hydrate.

Because of imbalanced structure of forages and grains across studies, meta-analytical approaches could not discern means for grain sources for ruminal starch digestibility as for total tract digestibilities in Table 1. Moreover, starch was rarely measured with earlier omasal studies; when starch was measured, the early studies were represented more by grain sources other than corn. If we go back to foundational studies with grains only added in studies with alfalfa as the sole forage (Firkins et al., 2001) and then updated (Ferraretto et al., 2013), comparable relationships can be applied for ruminal starch digestibility coefficients as in the total tract. The latter paper distinguished total starch digestibility by corn particle size (Table 1). Although not well represented in studies estimating ruminal digestibility, the inverse relationship between increasing ruminal starch digestibility by grain processing and

decreasing ruminal NDFD (Firkins et al., 2001) was confirmed recently with omasal sampling (Shipandeni et al., 2023).

Some researchers have treated grain with alpha amylase in efforts to increase ruminal (and possibly total tract) starch digestibility with decreasing dietary concentration of starch. An overall improvement of 0.5% digestibility is modest but probably depends on various conditions that can be more effective (Pech-Cervantes et al., 2022). Further research is needed for ruminal digestibility. Ideally, we would try to shift starch digestibility to the small intestine to limit negative effects of starch on NDFD, but compensatory starch digestion in the small intestine might be limited for ruminants (Moharrery et al., 2014; Harmon and Swanson, 2020).

One of the main physiological limitations is the negative associative effects of starch degradation on NDFD. Using treatment means (Ferraretto et al., 2013) and within-cow comparisons (de Souza et al., 2018), each 1% unit increase in starch decreases total tract NDF digestibility about 0.5 to 0.6% unit. Of course, starch is about twice the digestibility as NDF, so each 1% increase in starch is actually only ~ 0.75% net gain (subtracting the depressed NDFD). The carbohydrate chapter in NASEM (2021) describes how starch and byproduct fiber should be included when forage NDF is decreased. Although we need enough starch to optimize microbial protein, increasing starch beyond this sliding optimum decreases efficiency of microbial protein synthesis (Firkins et al., 2007; Hackmann and Firkins, 2015b). Microbes compete for substrate, especially for starch, whereas surface area probably limits their access to fiber. Therefore, with higher starch diets, rumen-degraded protein **(RDP)** is even more essential because the major proteolytics are also amylolytic and prioritize ammonia and amino acids for their own needs rather than sharing with cellulolytics (Firkins et al., 2025). Even with more RDP, traditional meta-analyses have documented that increasing ruminal starch degradability often is not linearly associated with measurements of microbial protein (Firkins et al., 2001). In that review, increasing ruminal starch degradability through more aggressive grain processing did not increase measurements for microbial protein production because of decreased efficiency of microbial protein synthesis.

Since 2001, researchers shifted from duodenal to omasal sampling for animal handling purposes. As mentioned previously, there are limitations in representation for starch digestibility in omasal studies with different grain processing methods. However, NDFD has consistently been measured. In the meta-analysis of Huhtanen et al. (2010), ruminal NDFD was predicted to be 95 to 97% of total tract NDFD. Although authors have argued for omasal NDFD to explain the difference, there is limited support for that argument. The relatively few studies with cannulas in the ileum averaged 11.9% NDFD (Gressley et al., 2011), which of course would be far greater than that estimated by omasal sampling. These large differences among sampling approaches for ruminal digestibility introduced major statistical roadblocks.

To remove interactions of the study effect with other variables, an intercept shift was needed for predicted ruminal starch degradability and microbial protein production in NASEM (2021). The resulting equation used by NASEM (2021) included a squared effect of dietary crude protein **(CP)** for predicting ruminal NDFD, and the squared term was deemed as biasing predictions for NDFD when CP is high (Martineau et al., 2023). Although those authors noted an inflection at 27% CP above which NDFD became negative, Firkins and Lapierre

(2023) cautioned that 21.4% CP (mean plus 2 standard deviations in the derivation database) should be used as a maximum response to limit such a bias (the error from the squared term grows geometrically with increasing CP). Even 21% CP is excessive in practice unless animals are grazing lush pastures for which few measurements are available for modeling anyway. Even if not a problem in normal diets, this problem with CP2 term documents the challenges with meta-analyses when various dietary nutrients are not balanced across studies. We have limited mechanistic understanding of how dietary CP (probably a proxy for RDP, which rarely is measured) affects ruminal starch digestibility, NDFD, and microbial protein production in lactating cow studies.

Fibrolytic Bacteria Benefit from Preformed Amino Acids

The RDP in feeds has been estimated using solvents, in vitro, or in situ methods; even so, most studies in meta-analysis used library values based on those procedures. The lack of consistent measurement in lactating cows is in contrast with its importance. Peptide supply is a critical factor for bacteria degrading nonstructural carbohydrates (Van Amburgh et al., 2015), yet even peptides (as opposed to RDP) rarely have been measured owing to inconsistent measurements (Firkins et al., 2007). Reynal et al. (2007) opted for a molecular weight filter to assess a relatively large proportion of dietary amino acids **(AA)** passing in peptides <10 kDa molecular weight. Almost nothing has been done since. Although CNCPS version 6.5 modified peptide supply according to this information (Van Amburgh et al., 2015), our unpublished work suggests that fibrolytic bacteria (and NDFD) are responsive to peptide supply in continuous culture. Thus, more attention should be given to the need by cellulolytic bacteria to use preformed AA (Wallace et al., 2001). Three different cultures of cellulolytic bacteria increased cellular growth from small peptides and AA versus ammonia, particularly when grown on cellobiose (the repeating disaccharide in cellulose) compared with a source of pure cellulose (Atasoglu et al., 2001). However, that cellulose source is relatively crystalline and need not represent the type of cellulose in high quality forages or fibrous byproducts. Therefore, an important takeaway is that adequate RDP from true protein is needed to optimize NDFD.

Previously, the role of peptides was discussed as stimulating the amylolytic/proteolytic microbes but also potentially for cellulolytics—either directly for AA or indirectly to be provided with ammonia and branched-chain volatile fatty acids **(BCVFA)**. Peptides probably have a more nuanced role. Peptide concentration is a net of production and usage. Probably there is an optimum supply of peptides in which too little can limit NDFD, but too much peptide supply might limit NDFD (Jones et al., 1998); moreover, diets providing such high amounts of peptides would not be economical. On the other hand, preformed AA provided from adequate RDP (not excessive) increases efficiency of protein synthesis in most microbial cells. Reliance on blood urea-N for microbes in low RDP diets cannot make up for peptide needs.

Peptides have been studied in multiple ways over the past decades. However, studies dosing just protein in batch culture likely are biased by limiting carbohydrates to effectively convert those peptides into cells (Hackmann and Firkins, 2015b). When studied, increasing ammonia concentration usually inhibited peptidolysis as a feedback mechanism to avoid wasting valuable AA. However, in addition to ammonia, availability of all AA is probably sensed through branched-chain AA concentration inside cells (Firkins et al., 2024). Sensing both ammonia and probably a balance of leucine,

valine, and isoleucine helps queue bacteria to grow more rapidly and efficiently. Although lack of peptides or AA limited cellulose digestibility (Griswold et al., 1996), adequate ammonia is needed to support microbial growth and therefore peptidase activity (Griswold et al., 2003). Because of the central role of ammonia, its limitation must be managed first, whereas AA from peptides can then secondarily limit microbial growth.

Our unfolding research suggests ammonia limitation must always be prevented before supplemental BCVFA can be efficacious (Firkins et al., 2024). However, phenylalanine might be particularly limiting for the cellulolytic bacteria (Wallace et al., 2001) and likely is one reason why true RDP (i.e., not urea) must be adequate for optimum fiber digestibility and response to BCVFA supplementation. There are numerous papers in the literature showing inhibition of peptidases with increasing peptide supply, and supplemental BCVFA can both increase or decrease peptidase activity, depending on conditions (Firkins et al., 2024). Although RDP typically is estimated as the loss of protein through a bag or a filter, that does not verify complete breakdown of peptides. Many papers suggest that peptidolysis is not constant such that peptides probably contribute more than projected to rumen-undegraded protein. Across different protein sources, there was an average of 10% of total AA flow from the rumen being of soluble dietary origin (Reynal et al., 2007), which is higher than the 6.4% of the A fraction passing in NASEM (2021). Ruminal cellulolytics need adequate RDP (more specifically peptides for breakdown to AA) to optimize microbial protein synthesis.

Peptidolysis and the Isoacids

If we add the layer that peptidolysis is not a constant, then we should be considering that the AA within RDP are not degraded at constant proportions. Proline, phenylalanine, and the branched-chain AA are candidates for increasing outflow relative to degradation (Chen et al., 1987); these all have important roles in metabolism by cellulolytic bacteria (Wallace et al., 2001). These results support our takeaway that adequate ruminal ammonia plus amino-N seem to be a predicate for the efficacy of BCVFA to stimulate NDFD (Firkins et al., 2024). Our current research in progress supports this conclusion. In a recent study (Redoy et al., 2025), adding urea at 0.19% of dietary DM (about 0.5% CP basis) in diets containing 10.3 to 10.5% total RDP increased NDFD, but the response was greatest with cows fed a higher forage (20.7% forage NDF) than lower forage (16.6% forage NDF; not accounting for whole cottonseed) in the diet. Milk fat yield was increased when BCVFA were added to high forage diets, but average daily gain was increased in higher grain diets. Our unpublished research agrees with their results and suggests that a shift toward average daily gain would be more pronounced with primiparous than multiparous cows. Using isotopes of the BCVFA, increasing forage increased isotope recovery in bacteria from continuous cultures administered higher forage diets, emphasizing the need by cellulolytic bacteria for BCVFA (Mitchell et al., 2023b). Thus, for BCVFA to have the most opportunity to maximize NDFD, there must be both adequate ammonia and adequate provision of preformed AA in the RDP.

Cellulose is all glucose but varies considerably in its crystallinity and interactions with other cell wall components. Hemicellulose, pectin, and other soluble fibers vary considerably in chemical makeup and bonding. Thus, cellulolytics are specialists with respect to how they use crystalline cellulose but still hydrolyze hemicellulose primarily to gain access to the long strands of cellulose. In contrast, hemicellulolytic

and pectinolytic bacteria tend to be generalists that work on a variety of substrates. Emerging evidence is that many of these generalists shift their gene expression of enzymes needed as these non-cellulose substrates are changing among diets or over time since the last meal (Firkins et al., 2025). Some of these generalists can also use starch, and many are active protein degraders. Thus, a balanced consortium of microbes fosters cellulolytics (which do not degrade protein) at the base, which opens up room for others and even "shares" oligosaccharides. Hemicellulolytics share in this niche for carbohydrate and help to provide protein degradation end-products, such as BCVFA and ammonia. In contrast, an unbalanced population, especially with an overly high abundance of starch degraders, allows excessive growth of "selfish" bacteria. Low pH and lack of other growth factors further tilt abundance toward the selfish ones. The cycle progresses with high starch or poor feedbunk management as unbalanced populations lose potential NDFD—even before a dysbalanced population would be promoting subacute acidosis.

Pectin has long been ignored in research because it has been assumed to be nearly completely degradable. However, pectin differs in its chemical structure, proximity in the plant cell wall, and in its role in the microbial consortium. Growing evidence suggests that pectinolytic bacteria include generalists and specialists. Some generalists are closely related to hemicellulolytics (e.g., *Prevotella* or *Butyrivibrio*). Other pectinolytic specialists include the corkscrew-shaped *Treponema*, which vortexes non-motile cellulolytics into newly ingested fiber particles and often shares their requirement for BCVFA as growth factors (Firkins et al., 2024). The latter have been routinely characterized as partners with the specialist cellulolytic bacteria. Soluble fiber from citrus pulp has been studied even less than that from forage but probably fall into a similar camp. Those microbes growing on soluble fiber probably also benefit from having adequate peptides or BCVFA derived from them to maximize efficiency of microbial protein synthesis.

Replacing Starch with Sugars (or Oligosaccharides)

Although there are procedures for estimating the soluble fiber concentration, the most common approach in research is by residual organic matter **(rOM)**. This pool is derived by difference of organic matter – CP – NDF – lipid – (starch+sugar). If done right, rOM is repeatable and very highly degradable (Tebbe et al., 2017). In NASEM, the rOM was somewhat also an error pool, i.e., inflating variation by combining errors from all of the analyses. We could not derive an equation using rOM, but plotting rOM alongside the predicted microbial protein had no residual patterns. In other words, rOM is probably baked into the equation in normal diets, although diets low in NDF and starch (i.e., high in soluble fiber) might predict lower microbial protein than actual.

Sugars can be from many different sources. Both citrus pulp or byproducts from sugar processing (both cane and sugar beet) contain free sugars and so are categorized as sugar sources. However, they also can contain significant oligosaccharides measured as soluble fiber. Is it the sugar or the soluble fiber? We do not really know why sugars sometimes improve NDFD (Firkins et al., 2025). However, any potential improvement would likely be negated if provided with high inclusion of starch, and moderate starch diets likely are needed for any benefit in increased milk fat secretion (Oba, 2011). There are few studies, and they are inconsistent with respect to microbial N production from supplemental sugars. Feeding

sugars can decrease ammonia or BCVFA concentrations (Firkins et al., 2006). Part of the sugar response could be through maintaining a basal prevalence of microbes using lactic acid; some of these might be able to use or benefit from increased AA availability.

Fatty Acid Digestibility

Firkins et al. (2025) emphasized potential roles for moderate amounts of palmitic and oleic acids to fibrolytic microbes. Numerous studies from Dr. Lock at MSU have noted a consistent improvement in NDFD from palmitic and probably with an appropriate blend of oleic acid for FA digestibility but also for NDFD. After all, palmitic acid fed at > 90% of total FA would have poor digestibility (NASEM, 2021; Table 2). The relationship between fat to stimulate NDFD could stimulate microbial growth, which could enhance flow of FA that help disperse palmitic acid in micelles. Although primarily containing palmitic and stearic acids, bacteria also contain oleic and especially branched-chain FA that would escape to the small intestine for production of monoglycerides and phospholipids. The FA from bacteria tend to be more highly digestible than dietary or endogenous FA (Schmidely et al., 2008). Protozoa are even better at sequestering unsaturated FA than are bacteria, which would improve FA digestibility not just of the microbes (5 to 10% FA) but also of dietary FA.

Fat sources are grouped into classes in NASEM (2021) according to the work by Daley et al. (2020) as shown in Table 2. However, there are many potential modifying influences on FA digestibility within those fat classes. Perhaps with a threshold prior to depression, supplemental stearic acid likely is less digestible than supplemental palmitic acid (Western et al., 2020). Blending of oleic acid with stearic acid might improve 18C FA digestibility as it does for 16C FA digestibility (de Souza et al., 2021). Those authors also noted how palmitic acid improved NDFD (to be discussed subsequently), but decreasing palmitic:oleic (i.e., increasing oleic) also increased NDFD. Oleic acid (but also other amphiphilic lipids such as those in microbial membranes) likely help to improve FA emulsion and absorption from the small intestine. However, when ascertained on a total 18C basis to account for ruminal biohydrogenation, oleic acid substitution for stearic acid is hard to distinguish the role of oleic acid on emulsion compared with oleic simply being more digestible than stearic. However, Prom et al. (2021) infused oleic acid into the abomasum to document its ability to improve FA digestibility, but of course biohydrogenation also was circumvented, so infusion would provide much more oleic acid than from the diet for reaching the duodenum. Despite some mixed responses in the literature, most reports show palmitic acid to be more digestible than stearic when mixed with oleic in appropriate ratios.

Oilseeds have unsaturated FA but also can be influenced by physical properties. Processing of whole cottonseed **(WCS)** can release oil and increase risk for milk fat depression; whereas ,the fiber is highly effective for stimulation of rumination even if processed. To prevent ruminal effects on NDFD, though, WCS is typically not processed. Most likely the FA digestibility in unprocessed WCS is lower than that in basal feeds because of decreased release of oils from the seed. Bales et al. (2024) noted increased 16C digestibility but decreased 18C digestibility with increasing supplementation of WCS. In contrast, WCS products did not affect 16C but increased 18C digestibility (Reveneau et al., 2005). Whole soybeans generally have been grouped with other oilseeds (WCS, rapeseed, sunflower seed, etc.) and can have lower (Boerman et al., 2015) or comparable (Daley et al., 2020) FA digestibility compared with FA in basal diets. Most likely, a moderate rolling

of oilseeds helps to disperse oil for optimal FA digestibility and with minimal disruption to the rumen. However, feeding free oil from high oleic soybeans improved FA digestibility but tended to decrease NDF digestibility (Hanno et al., 2024). Most studies show that, if we avoid excessive free oil in the rumen, there will not be a depression in NDFD that is noted with higher inclusion. Although limited in research, increasing oleic acid and decreasing linoleic acid should not decrease FA digestibility of oilseeds.

Some Fats can BENEFIT Ruminal Fiber Digestibility and Microbial Protein Synthesis

Except for excessive doses of medium chain FA (lauric and myristic), fat probably has a minimal negative effect on NDFD (and therefore microbial N) unless fed at high enough inclusion rates to promote milk fat depression or depress DMI (Weld and Armentano, 2017), which should be avoided anyway. That meta-analysis suggested no benefit for palmitic supplements, but many of those palmitic supplements were from triglycerides. In contrast, after excluding all triglyceride sources, the free palmitic acid supplements averaged a 4.5% unit increase in NDFD (dos Santos Neto et al., 2021). However, as discussed below, palmitic acid should not be fed alone and typically is included with oleic acid.

Fibrolytic bacteria probably can pull in exogenous FA and activate the FA for conversion into phospholipids. In order to degrade fiber substrates, the specialist fibrolytics have unique and important enzyme complexes on their exterior (Firkins et al., 2024; Firkins et al., 2025). However, those enzymes must be synthesized intracellularly before being translocated through the cytosolic membrane prior to assembly into multi-enzyme complexes. In a "Goldilocks" approach, membranes must be fluid enough for transferring intact proteins yet rigid enough to anchor those complex factories to the cell wall. Cellulolytic bacteria vary in how they use these extracellular proteins, but there is no doubt that they are specialists because of them. However, even some bacteria degrading hemicellulose and resistant starch have critical assemblies that are on the exterior of the cell or in between the two membranes of gram-negatives.

As important as process is, we still do not know but can assume membrane fluidity is sensed and fatty acid synthesis adjusted in response to changes in ruminal conditions (Hackmann and Firkins, 2015a; Firkins et al., 2025). Because bacteria cannot desaturate FA anaerobically, they must add more fluidizing (unsaturated or branched-chain FA) as needed to be in a proper ratio with the stiffening saturated FA. The fibrolytics need much more branched-chain FA than those degrading starch (Firkins et al., 2024). However, the most well characterized biohydrogenating bacteria in the butyrivibrio group (genera *Butyrivibrio* and *Pseudobutyrivibrio*; primarily using hemicellulose) are particularly enriched in branched-chain FA (Hackmann and Firkins, 2015a). They also have low concentrations of stearic acid, and some have higher palmitic acid (opposite of most bacteria). Strains of the cellulolytic bacteria *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* also are high in palmitic and branched-chain FA but low in stearic; unfortunately, oleic was not recorded (Saluzzi et al., 1993).

Anaerobes do not store and use FA as fuel as do the aerobes, so decrease in chain length from 18 to 16 carbons appears limited. As explained above, palmitic acid supplements are typically combined with some oleic acid, which aids in intestinal absorption, but the same practice probably also balances uptake of rigidity (palmitic) and fluidity (oleic and the branched-

chain FA). Providing palmitic acid in the free form probably helps intestinal absorption but also makes this fat source more rumen-active, so again what is good for the cow is good for her microbes. Oleic is far more soluble in the rumen; inconsistent responses of oleic acid on ruminal NDFD (de Souza et al., 2021) could be a result of over-supplementation on an amount basis rather than on a rumen-available basis. If oleic acid is provided in supplemental fats and used by bacteria, there does not seem to be any lessening of the need for BCVFA (Mitchell et al., 2023a). 2-Methylbutyrate in particular seems particularly important followed by isobutyrate for plasmalogen synthesis, even when corn oil was supplemented.

The role of fats on microbial protein supply has been considered in multiple ways (Hanigan et al., 2021). Twenty years ago, fat was thought to dilute degradable carbohydrate and decrease dietary energy to support microbial protein synthesis. After studies showed no decrease in microbial protein production, fat was assumed to improve efficiency of microbial protein synthesis either by sparing carbon that would have been used for FA synthesis to be used for other purposes or else through decreased protozoal numbers and intraruminal recycling of microbial protein. More recently, those authors noted neither benefit nor detriment from dietary fat. However, in meta-analyses, each factor is always accounted for at the average of each other factor in a model. Fat can sometimes decrease DMI, which obviously would decrease microbial protein production because of lower carbohydrate and RDP supplies. Moreover, grouping FA as they did without considering FA profile and as free versus esterified FA could prevent any potential benefit.

Microbial Protein for Dairy Cattle

NASEM (2021) predicts microbial protein synthesis using predicted ruminal starch and NDFD and RDP. All of these were predictions from standard dietary analyses, which has limitations because some of these were predicted (including all of the RDP). NASEM was derived using in vivo data, whereas many models are being evaluated against in vivo data; therefore, users need to understand differences between those data derived using omasal versus duodenal sampling. For example, both the 2001 NRC (Broderick et al., 2010) and CNCPS version 6.5 (Van Amburgh et al., 2015) were evaluated against omasal flow data. However, NRC (2001) had no source data using omasal sampling. The evidence against duodenal sampling using a single marker is, in fact, not particularly robust (Firkins et al., 1998). However, when collecting samples from the omasal canal, the more complicated triple marker approach is needed. Almost no studies include both duodenal and omasal sampling; of those, there is a clear distinction in the stable isotope of nitrogen (N; i.e., ^{15}N) that is associated with the higher microbial N flow for omasal sampling than for duodenal sampling (Martineau et al., 2023). Therefore, is it the flow method and its markers or is it the microbial sampling and microbial markers? If there is no known "gold standard" approach to measuring microbial protein supply (and therefore the nonammonia-nonmicrobial N supply that standardized RUP measurements), we are relying on relative comparisons. For NASEM (2021), the intercept shift for higher microbial and lower nonmicrobial-nonammonia N for omasal sampling (~17.6% of treatment means) must be fixed in time. As more papers are published using omasal (or reticular) sampling, the NASEM (2021) model will continue to be increasingly "biased" compared with this changing evaluation database.

Other key differences remain among models or their evaluation. Because of endemic problems with markers themselves and because markers don't differentiate between protein and fiber, there is no specific marker for RUP. Therefore, the passage rate was derived iteratively such that RUP, on average had no bias from all of the protein sources in all of the studies compared with measured nonammonia-nonmicrobial flows (corrected for endogenous protein) in the database (Hanigan et al., 2021). Another complicating factor is that a number of studies used in NASEM (2021) were excluded for experimental reasons (Roman-Garcia et al., 2016), whereas other researchers are not excluding these studies. For example, studies estimating ruminal outflow using pool size and passage rate overestimate flow. In contrast, studies with duodenal cannulas placed distal (after) the pyloric valve will not subtract enough endogenous protein. We weighted results for SEM, which often has not been done by others; using residuals analyses to assess bias cannot account for the weighting factor. When using standard fitting procedures, residuals analysis from the same source data can NOT yield a mean bias, which has been included in some papers purportedly using most of the same source data. The point is that evaluations are continuing to arise, but their "bias" for NASEM (2021) might be data or procedural differences, not flaws in the prediction equation. For real gains to be made in the future, we need more data for ruminal NDF, starch, and rOM digestibilities combined with better diet descriptors, such as corn particle size, derivation of rOM without double counting protein in fibrous fractions, reporting of corn silage DM, and reporting of both lignin and uNDF for forages; otherwise, predictions will continue to be centralized toward the dataset means and be less useful for field conditions.

Conclusions

Feeding high corn is not necessarily a cheap way to provide protein to the cow because of decreased NDFD and efficiency of microbial protein synthesis. If corn is cheap and high RUP protein sources are relatively expensive, then maybe feeding higher amounts of corn would be a good option. However, if high RUP protein sources are relatively expensive per unit of delivered metabolizable AA, we should be maximizing NDFD, DMI, and microbial protein before the more expensive protein is used to more specifically moderate metabolizable AA profiles of duodenal digesta. Maintaining ruminal pH with appropriate forage particle size and feeding management while considering sugars (and soluble fiber) should improve ruminal digestibility and supply of energy for microbial protein synthesis. However, we need to remember that only about half of the starch suppression of ruminal NDFD is a result of pH, whereas the non-pH depression of NDFD is likely a result of insufficient growth factors for cellulolytics. We need to provide adequate RDP when considering supplementation of isoacids. Supplementation of palmitic/oleic fatty acid blends in moderate amounts can improve NDFD and perhaps also microbial protein supply. Using averaged values in NASEM software allows variability in individual on-farm conditions. Using more complicated models based on first-order rates such as current CNCPS platforms, could underpredict the negative effect of starch on NDFD (unless peptides are limited) and overpredict the supply of microbial protein. Thus, users should be aware of the mechanisms discussed in this paper to help troubleshoot or fine-tune rations.

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Table 1. Apparent total tract starch digestibility of cereal grains (%; NASEM, 2021).

Default	91
Corn grain, dry < 1250 µm	92
Corn grain, dry 1500-3250 µm	89
Corn grain, dry > 3250 µm	77
Corn grain, high-moisture < 2000 µm	96
Corn grain, high-moisture < 2500 µm	90
Corn grain, steam-flaked	94
Sorghum grain, dry, ground	83
Sorghum grain, steam-flaked	94
Corn silage, < 30% DM	91
Corn silage, 32-37% DM	89
Corn silage, > 40% DM	85
Grain sorghum silage	85
Barley, steam-rolled	94
Barley, ground	91
Wheat	93

Table 2. Apparent total tract fatty acid digestibility (%; NASEM, 2021)

Common feeds	73
Oilseeds	73
Oil	70
Blended triglyceride	63
Tallow triglyceride	68
Saturated fatty acid-enriched triglycerides	61
Extensively saturated triglycerides	44
Calcium salts of palm fatty acids	76
Saturated fatty acid-enriched non-esterified fatty acid	69
Palmitic acid, ~85% of total	73
Palmitic or stearic acid, > 90% of total	31