Taking the Stink Out of Branched Chain VFAs
Capturing Targeted Nutrient Feeding through Modeling

P. Andrew LaPierre and M.E. Van Amburgh
Department of Animal Science
Cornell University

Introduction

What was once old, is new again. And perhaps what's the best thing about this freshening up, is the lack of odor associated with it. Having never personally experienced the odor that kept many farm families at odds with each other, testimonials are still rather vivid whenever the word 'IsoPlus' is whispered in a dairy industry crowd. Previously developed in the 1980s as a byproduct of film making, the FDA had approved IsoPlus as a feed additive for dairy cattle diets. Claims of 'four to six pounds more milk per cow per day with minimal changes to their diet' can still be found with a quick YouTube search. The product, however, was short lived on the market (One could imagine the kitchen table conversations involving IsoPlus), yet the biological relevance of branched chain VFAs (BCVFA) is foundational when discussing the proper functionality of the rumen microbiome. Recent product development has blown the dust off this technology, taking a fresh approach to masking the odor surrounding these volatile compounds all while maintaining their mode of action. Obviously, dietary formulation has come a long way over the last thirty years, but with ever-increasing pressures for balancing nutrient supplies to boost productive efficiencies and minimize excessive nutrient excretion, a case for feeding exogenous isoacids can still be made. Most dietary formulation model, unfortunately, would not appropriately predict the effects isoacid feeding might have within the rumen environment, particularly in circumstances where they would be warranted. This paper aims to make a case where updates could be made to the Cornell Net Carbohydrate and Protein System (CNCPS) to better represent the effects of meeting this requirement would do to fiber digestibility and productive efficiency.

Where do BCVFA fit in rumen metabolism?

It has been long known that cellulolytic bacteria, including *Fibrobacter succinogenes, Ruminococcus albus,* and *Ruminococcus flavefaciens,* exhibit requirements for BCVFA (Andries et al., 1987) with the intention of using them to synthesize branch chain amino acids (BCAA) through reductive carboxylation (Allison et al., 1962b, Allison, 1969, Robinson and Allison, 1969) or branch chain fatty acids (BCFA) through chain elongation (Allison et al., 1962a). These cellulolytic bacteria lack the ability to r intercellularly transport or synthesize one or more BCAA necessary for growth and proliferation (Mitchell et al., 2023a). The majority of BCVFA found in the rumen are by-products of BCAA metabolism from other dominant species of bacteria, whereby, the deamination of the amino group or transamination of the corresponding keto acid occurs. Ironically, it has been observed that the amylolytic bacteria, *Prevotella ruminicola,* which does not require BCVFA to synthesize BCAA, will preferentially carboxylate them back to

BCAA, down regulating de novo BCAA production and reducing cross feeding to bacteria who cannot synthesize BCAA without BCVFA as the precursor (Allison et al., 1984). A strong argument can be made that the reduction in aNDFom digestibility observed in dairy cattle with higher levels of dietary starch (de Souza et al., 2018) is due to this resource competition. Bacteria that digest starch have the capacity to take up peptides and amino acids (Russell and Sniffen, 1984; Chen et al., 1987) which provides them a competitive advantage over fiber digesting bacteria which can only utilize ammonia and have a much slower growth rate (Bryant, 1973). Exogenous supplementation of these BCVFA has shown improved aNDFom disappearance in batch cultures (Russell and Sniffen, 1984, Cummins and Papas, 1985, Roman-Garcia et al., 2021a), providing necessary growth factors for primary colonizers in fibrotic material; however, in diets where true rumen degraded protein (RDP less ammonia concentration) is provided in sufficient amounts, no such improvements in disappearance are observed (Copelin et al., 2021). Further, supplying these iso-acids has demonstrated improvements in dry matter intake, milk volume (Andries et al., 1987), and milk components (Wang et al., 2019).

Where do BCVFA fit in diet formulation modeling?

Under the current structure of CNCPS v6.5.5 (Fox et al., 2004, Tylutki et al., 2008, Van Amburgh et al., 2015) predictions for the ruminal disappearance of carbohydrate and proteins adhere to first order kinetics which are calculated using rates of degradation and passage for each ingredient in percent per hour, establishing the maximum potential with which a particular feed fraction can be degraded. Additionally, a microbial yield coefficient is also calculated for each carbohydrate fraction in the diet, using the intrinsic rate of degradation for each feed ingredient in percent per hour, the maintenance rate of bacteria in grams of CHO per gram of bacteria, and growth potential of that bacteria in grams of bacteria per gram of CHO. Each yield coefficient is applied to the corresponding feed fraction to estimate microbial yield for a given feed. Summation of the microbial yields for CHO A2, A3, A4, B1, and B2 feed fractions represents the potential non-fiber carbohydrate (NFC) degrading bacteria pool whereas CHO B3 is the sole carbohydrate fraction used to estimate the potential fiber carbohydrate (FC) degrading bacteria pool size (Fox et al., 2004). These estimates of carbohydrate disappearance and microbial pool size are predicated on the assumption that the rumen is not limited in other growth factors, especially nitrogenous substrates. The CNCPS estimates total available rumen N by summating dietary ammonia intake (PRO A1), recycled N (Recktenwald et al., 2014), and dietary peptides and degraded feed N from microbial action. This N is pooled together to estimate the potential bacterial growth based on the N content of both NFC and FC degrading bacteria (Russell et al., 1992). This pool is then appropriated to each ingredient within the diet based on the proportion that each feed ingredient contributes to the total degraded carbohydrate (i.e., if feed ingredient one contributed 15% of the degrade carbohydrate from the diet, then 15% of the total nitrogen pool is appropriated to the degradation of that ingredient). Taken with the carbohydrate allowable microbial yields, the system considers whether the amount of N appropriated to each feed ingredient is sufficient to achieve the potential carbohydrate degradation calculated. If the predicted allowable N pool is inadequate to complement the potential carbohydrate degradation, the system will restrict the level of carbohydrate degraded, prioritizing a limitation of fiber carbohydrate degradation first, based on metabolic activities of NFC and FC bacteria. Further, this limitation in available N and degraded carbohydrates will depress predicted bacterial flow, causing a reduction in metabolizable protein (MP) which would hinder productivity of the animal.

As the industry looks to reduce protein feeding in lactating diets, the likelihood that a nutritionist would encounter a scenario where predicted rumen N is not sufficient to meet potential carbohydrate degradation is greater than previously seen. When such dietary scenarios present themselves, it is imperative to recognize certain limitations under the current CNCPS predictions. Presently, the CNCPS amalgamates all nitrogenous substrates into one N pool, considering them all equal when reconciling the necessary substrates for proper microbial metabolism, proliferation, and feed degradation. As such, in instances where nitrogen supply is low, inclusion of dietary urea may be used as a method to improve rumen total N pool size, giving the appearance in the model that sufficient N is present to realize potential carbohydrate degradation and microbial yield. This solution creates a fallacy, as rumen ammonia supply is likely not the prevailing cause for this limitation in carbohydrate degradation. Branch chain amino acids, which are currently not considered in the rumen N pool, but, because they are precursors for BCVFA, also have a unique carbon backbone used for other metabolic processes, become limited in scenarios when dietary protein is concomitantly reduced. This presents an opportunity to disaggregate the rumen N pool, allowing for the consideration of BCAA/BCVFA sufficiency.

To account for BCAA/BCVFA adequacy in the CNCPS, an approach similar to Tedeschi et al. (2000) has been integrated into the CNCPS and its preliminary results are shown below. Like the predicted supply of other nutrients, the user defined feed chemistry of dietary ingredients will be used to estimate the supply of BCAA and any exogenous sources of BCVFA. Because oxidative deamination of BCAA by microbes is still the primary source of ruminal BCVFA (Allison et al., 1962b), the rate of BCAA deamination, release of BCVFA by bacteria, and assimilation of BCVFA by other bacteria are all important considerations in predicting the status of BCVFA within the rumen. At present, work from Atasoglu et al. (2004) is being used to estimate the rate in which BCAA are oxidatively deaminated to BCVFA by amylolytic bacteria and the proportion of excreted BCVFA which are taken up by fibrolytic bacteria. The primary fates of BCVFA are defined in the CNCPS as BCAA or BCFA, with the understanding that a small proportion of BCVFA may be used for branch chain keto acid production (Firkins, 2021). Bacterial protein content and amino acid profile data already exists within the CNCPS and is used to predict the amount of bacterial outflow from the rumen (Russell et al., 1992); however, only the bacterial fat content and not the profile of fatty acids, and further BCFA, is expressed in the CNCPS. As such, fatty acid profile data from Vlaeminck et al. (2006) has been used to profile the fatty acid composition of bacteria and identifying which of these fatty acids are classified as BCFA to establish recommended feeding rates. Taking all these predictions together, the supply of BCVFA from the conditions specified by the user are used to estimate the allowable bacterial growth from BCAA/BCVFA supply. Similar to the concept of the total rumen N pool, in situations where rumen degraded BCAA and exogenous BCVFA are not sufficient enough to meet the potential microbial growth from degraded carbohydrates, the system will restrict carbohydrate degradation and subsequent microbial growth based on what is supplied. Independent of the rumen N pool, a user of the CNCPS would be able to troubleshoot whether a low protein diet was limited in either rumen N, BCAA/BCVFA, or a combination of both. As such, limitation born from BCVFA deficiencies cannot be overcome with the supplementation of urea and will only be reconciled when either true RDP with an appropriate level of BCAA or a concomitant substitution of exogenous BCVFA are provided.

To test this approach, a data set consisting of 1,352 treatment means (Table 1) ranging in true RDP, starch, and fiber content were evaluated using the amended version of the CNCPS. This evaluation identified which of these diets had no substrate limitation when degrading the potentially degradable carbohydrate substrate from the diet, while flagging which of them were limited by either rumen N or by the BCAA/BCVFA. These nutrients (BCAA/BCVFA) are being used synonymously because limited data exists to identify whether the limitation lies with BCAA or BCVFA as one can be made from the other and they are cycled extensively (Firkins, 2021). Of the diets which were flagged for a BCAA/BCVFA limitation, an exogenous source of BCVFA was included in each of the diets at a rate which would overcome this limitation. The diets were rerun through the CNCPS, and the results collected. The primary responses that the CNCPS predicted was improvements to aNDFom digestibility (NDFD), MP from bacterial sources, and potential milk yield (Table 2). Multiple experiments conducted in continuous cultures indicated a 5.0% (Roman-Garcia et al., 2021b) and 7.6% (Mitchell et al., 2023b) improvement in NDFD when BCVFA were supplemented under various dietary conditions. Average improvements from this evaluation suggested a 3.7% increase in NDFD, complementing the results observed in the literature. Consequently, the improvements in NDFD resulted in an increase in MP from bacteria, averaging 32.1 g, and a concomitant increase in predicted milk of approximately1.1 kg.

Responses to the supplementation of exogenous BCVFA in the revised CNCPS are graphically depicted in Figure 1, 2 and 3. Each figure displays all diets which were either limited in N, limited in BCAA/BCVFA, or showed no limitation when evaluating the potentially degradable carbohydrate from the diet. The data in Figure 1 describes the predicted differences when comparing this response to the diets starch and potentially digestible (pd) aNDFom content, whereas Figures 2 and 3 show the describes similar differences when related to either starch and true RDP or fiber content and true RDP content, respectively. Not surprisingly, nearly all diets with a higher NDF content expressed a demand for BCVFA supplementation. Of these diets, those with greater starch content (>35% DM) showed an even greater increase in NDFD; however, nearly all dairy cattle are below this level of starch content. True RDP content was less of an influence on the demand for BCVFA when pdNDFom and starch content was considered, as diets across the entire range of true RDP were considered limited in BCVFA, particularly when pdNDFom content was high. It is worth noting that there were several higher fiber diets which were not flagged as being limited in BCVFA because the AA profile of the ingredients within the diet had a larger portion of BCAA available for rumen metabolism.

Table 1. Descriptive statistics for dataset used to evaluate BCVFA updates within CNCPS.

Parameter	n	Mean	Std	Min	Max
Dry Matter Intake	1325	18.7	4.1	6.9	30.4
Original Milk Production, kg		27.8	7.5	4.8	48.2
True RDP, % DM		9.9	1.6	5.2	19.5
Starch, % DM		24.3	9.8	0.6	56.4
aNDFom, % DM		33.6	6.9	14.8	62.6
pdNDFom, % DM		25.0	6.3	10.0	51.2

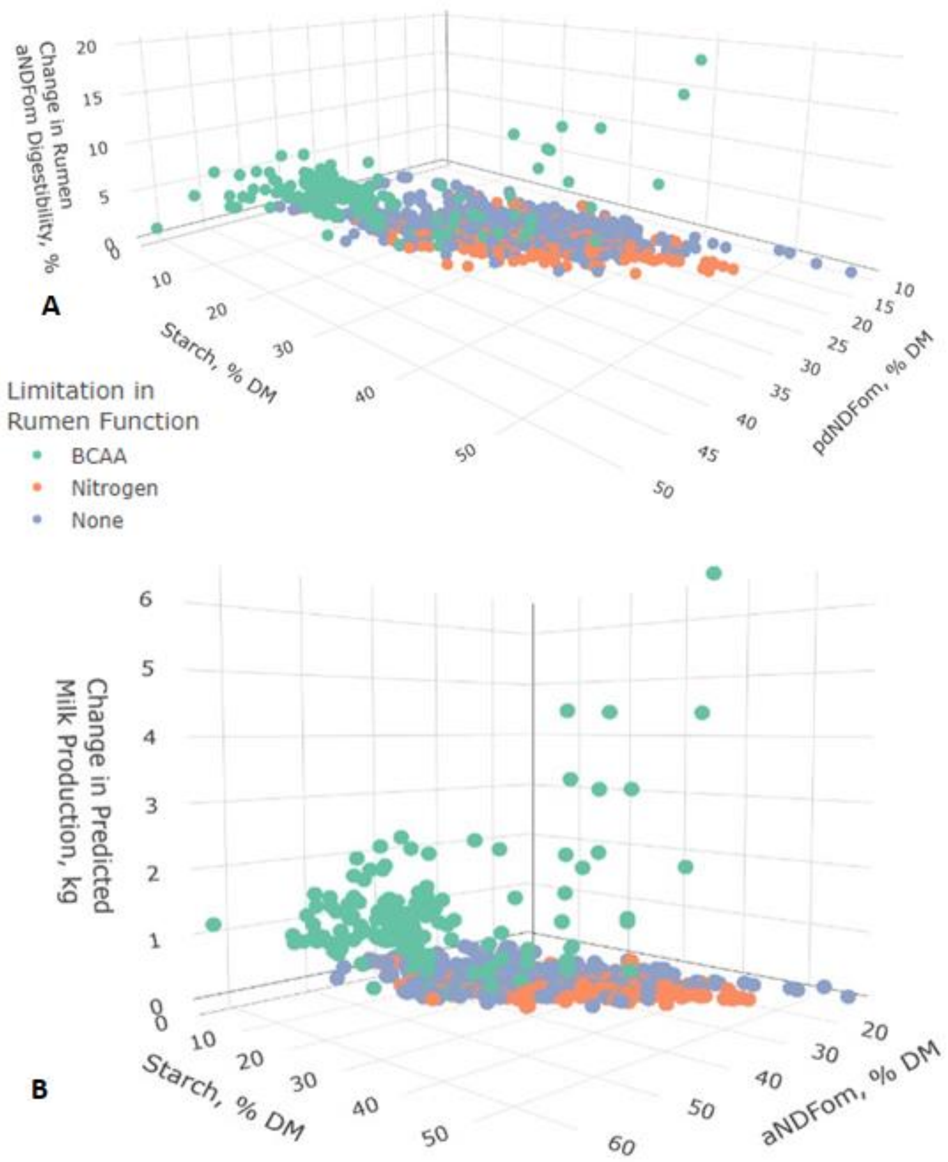

Figure 1A & B. Relationship between dietary starch content, fiber content, and changes in either predicted rumen fiber degradation or milk production with the addition of isoacids to diets which are either limited by N supply, BCAA supply, or are not limited by nitrogenous supply.

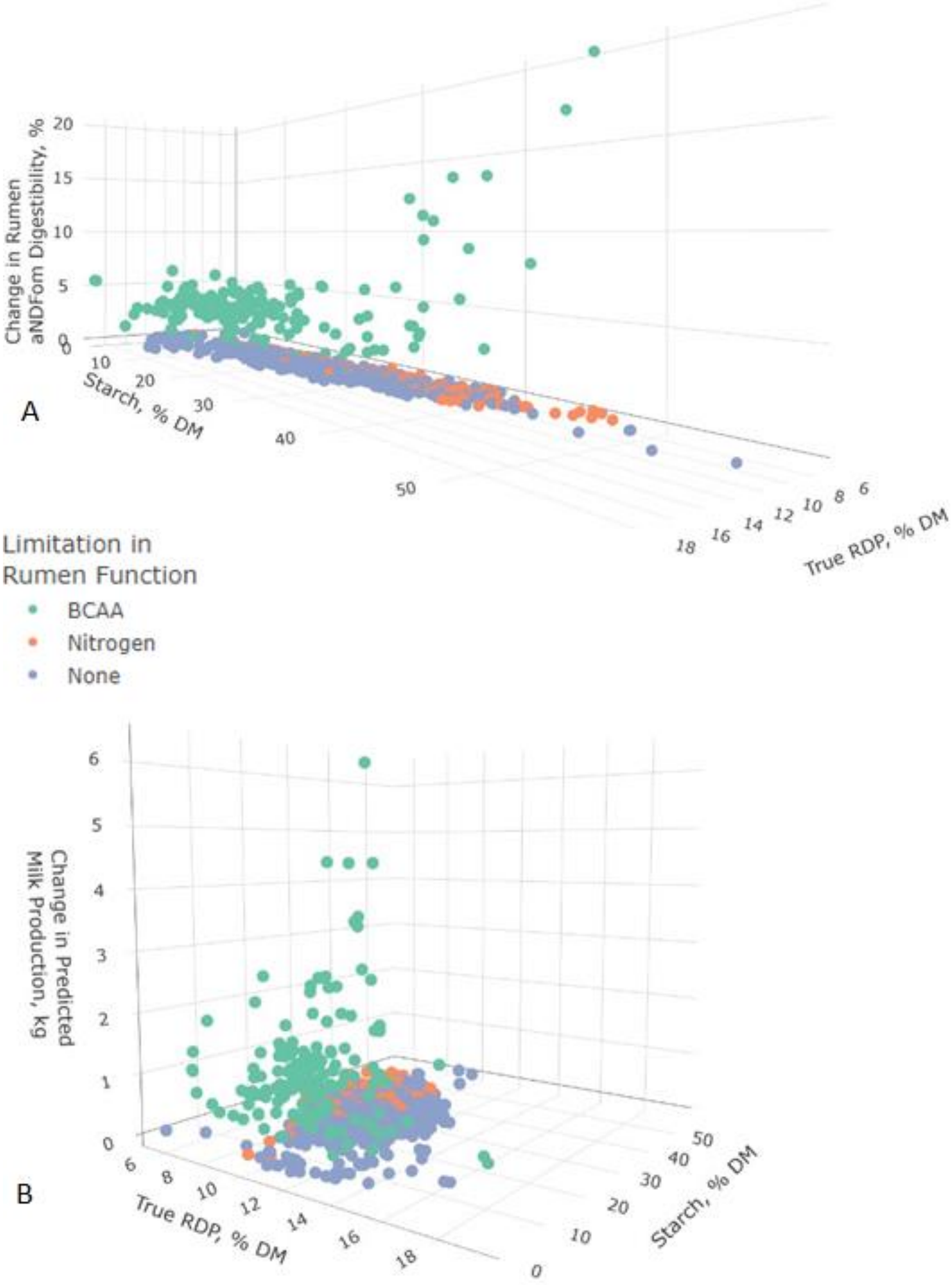

Figure 2A & B. Relationship between dietary starch content, true RDP content, and changes in either predicted rumen fiber degradation or milk production with the addition of isoacids to diets which are either limited by N supply, BCAA supply, or are not limited by nitrogenous supply.

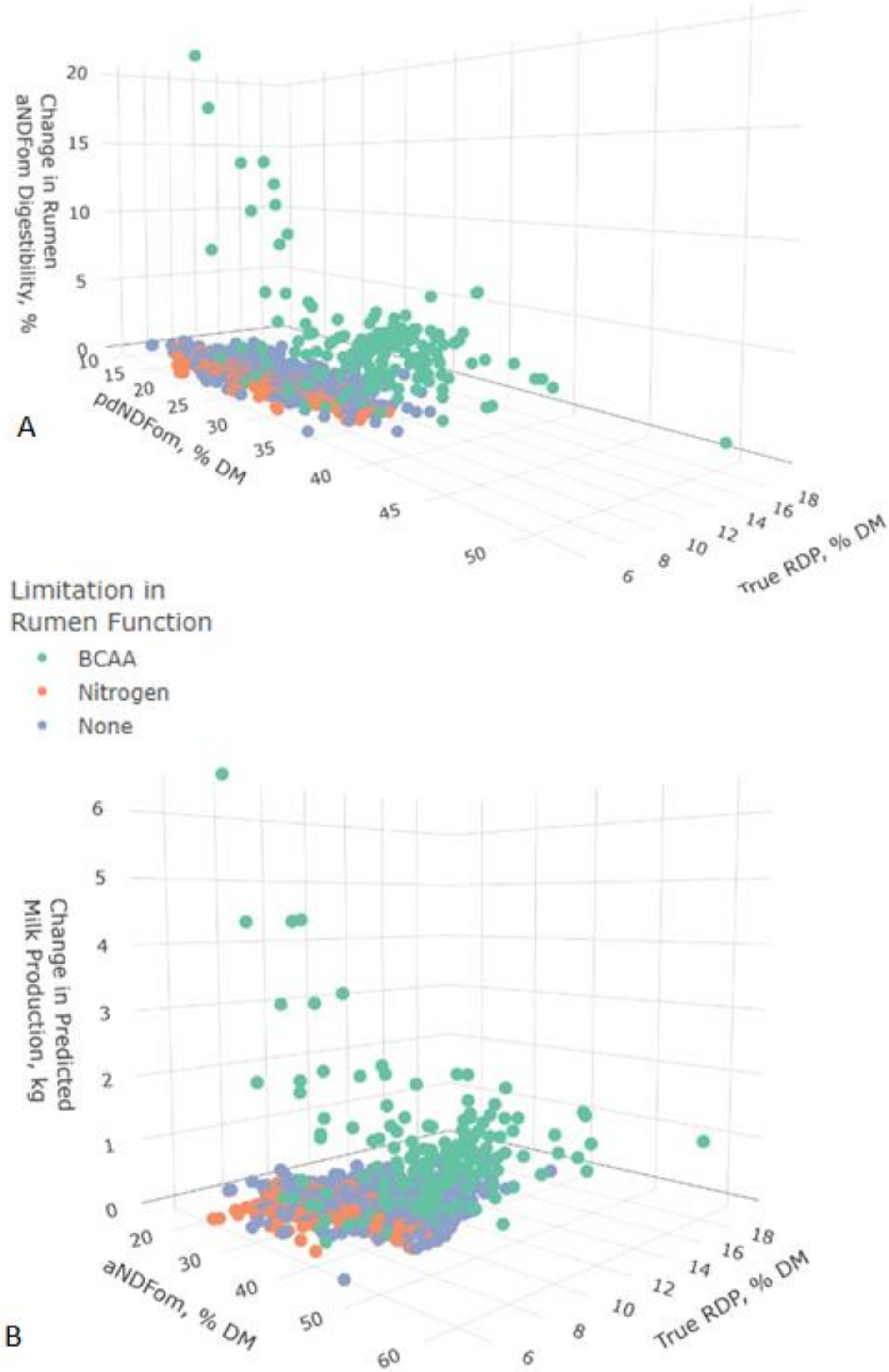

Figure 3A & B. Relationship between dietary fiber content, true RDP content, and changes in either predicted rumen fiber degradation or milk production with the addition of isoacids to diets which are either limited by N supply, BCAA supply, or are not limited by nitrogenous supply.

Table 2. Average predicted response and relationship to dietary aNDFom, starch and true rumen degradable protein for rumen aNDFom degradability, metabolizable protein sourced from bacteria, and milk production. Responses are a result of revisions to the CNCPS to better represent BCVFA supplementation.

		Regression Analysis				
Response to BCVFA Supplementation	**Average Response**	**R-Squared**	**Parameter**	**Estimate**	**Std Error**	***P*-Value**
Rumen aNDFom degradability, %	3.7	0.44	Intercept	6.46	2.852	0.02
			aNDFom	-0.098	0.0475	0.04
			Starch	0.092	0.0300	0.00
			True RDP	0.0248	0.0958	0.80
Bacterial MP, g	32.1	0.41	Intercept	-0.329	0.7987	0.68
			aNDFom	0.0216	0.0169	0.20
			Starch	0.0439	0.0089	< 0.01
			True RDP	0.0081	0.0299	0.79
Milk yield, kg	1.10	0.41	Intercept	0.440	0.9129	0.63
			aNDFom	0.0025	0.0152	0.87
			Starch	0.038	0.0096	0.00
			True RDP	0.0028	0.0307	0.93

Conclusions

The improvements discussed here serve as the first step toward a continued evolution to predicted more discrete nutrient interactions within the rumen that have real implications on fiber degradation, microbial yield, and cattle performance. As more research works to describe microbial metabolisms surrounding BCVFA, there will be efforts made to improve not only the predictions but also the structure surrounding the predictions, so that there will more discrete outputs from the CNCPS which will aid in diagnostic work surrounding lower protein diets and which nutrients may be hindering optimal digestibility and microbial efficiency.

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