Guide for Incorporation of Enteric Methane-Reducing Ingredients and Feed Additives into the Cornell Net Carbohydrate and Protein System

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Cornell University

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Approach to Report

In this document, the word "*must"* indicates that an action is mandatory, whereas "*should"* indicates that the action is suggested. "*May"* signifies a recommendation. "*Solution providers*" refers to developers of enteric methane inhibiting products (EMIPs).

Applicability

This guide is structured around the current architecture and data requirements of the Cornell Net Carbohydrate System (CNCPS). As the CNCPS and the underlying science continue to evolve, the criterium provided here may be subject to updates accordingly and does not represent fixed standards by CNCPS developers.

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Definitions

Active compounds: The identified compound that elicits the observed anti methanogenic effect in the ingredient or feed additive.

Animal performance: Milk production, milk composition, DMI, rate of gain.

Chemical profile: Nutrient profile of ingredient or feed additive as determined by wet chemistry analysis.

Crude protein (CP): An estimation of the amount of protein in an ingredient or feed additive through its nitrogen content.

Dry matter (DM): The portion of feed (diet, ingredient, additive) that does not contain water and is the common measure of intake for animals.

Dry matter intake (DMI): The amount of dry matter an animal consumes on a daily basis.

Energy corrected milk (ECM): Expresses milk yield as the amount of energy equivalent to milk containing a specific percent (%) fat and protein, using those values as a reference standard than fixed targets for composition.

Enteric methane-inhibiting products (EMIPs): A category of technologies that have properties that reduce the amount of enteric methane produced by ruminants. Includes but is not limited to ingredients and feed additives that are delivered to cattle in their diets.

In situ: Studies that conduct research within a specified environment of an organism.

In vitro: Studies that take parts of an organism to conduct research on that organism outside of its body.

In vivo: Studies that occur in a living organism.

Microbial nitrogen: The portion of nitrogen derived from passage of microbes from the rumen subject to digestion

Methane reduction factor: The estimated methane reductions achieved by EMIPs and best farming practices.

Neutral detergent fiber (NDF): A commonly utilized measure of fiber that includes the structural components of plant cell walls (hemicellulose, cellulose, and lignin).

Non-protein nitrogen (NPN): Nitrogen compounds that can contribute to the crude protein levels of the diet (urea is the most common)

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Omasal flow: The quantity and composition of digesta leaving the rumen and entering into the omasum.

Omasum: The third compartment of the four chambered ruminant stomach.

Organic matter (OM): The portion of feed does not contain moisture or ash, thus representing the energy-providing fraction.

Rate of degradation (kd): The speed at which feed fractions are degraded in the rumen.

Rate of passage (kp): The speed at which feed fractions leave the rumen.

Total tract digestibility: The proportion of a given nutrient that is digested and absorbed across the entire gastrointestinal tract, calculated as the difference between nutrient intake and nutrient excretion in feces relative to its intake.

Volatile fatty acid profile: The concentration of mainly acetic, propionic, and butyric acids produced via fermentation in the rumen.

Wet chemistry analysis (WCA): A set of analyses run with chemical solutions that quantify the nutrient profile of feed.

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Background

The Cornell Net Carbohydrate and Protein System (CNCPS) is a mechanistic, feed-centric model with a library of more than 900 ingredients and serves as a critical tool across most U.S. dairy operations to predict nutrient utilization and animal performance on given formulated diets. Standard ingredients in the library have undergone a consistent characterization process, including a detailed chemical analysis and digestibility assessment to determine the nutrients available to the animal when fed. These inputs inform the submodels that predict feed digestion and passage, performance outcomes, and associated energy losses, including enteric methane emissions.

Enteric methane-inhibiting products (EMIPs) in the form of feed ingredients or commercially available feed additives that provide enteric methane reductions when included in the diet are rapidly emerging as a mitigation tool to address the production of methane as a greenhouse gas from dairy and beef systems. However, the ease of adopting these tools remains a challenge due to the paucity of literature regarding their nutritional characterization, their full influence on rumen metabolism, ruminal adaptation following long-term supplementation, and potential interactions with other EMIPs and diverse dietary contexts. The CNCPS currently predicts enteric methane emissions through established energy-loss pathways and decades of submodel development. However, emerging EMIPs introduce a spectrum of non-nutritive effects on rumen metabolism that are still being quantified. While the CNCPS library is diverse, it does not yet include EMIPs as their biological effects are not fully quantified or readily parameterized in the model.

Inclusion of any new enteric methane-reducing feed ingredient or feed additive in the CNCPS requires thorough characterization that can typically be performed by a commercial lab. However, for the CNCPS to accurately predict methane abatement due to EMIP inclusion in the diet, a deeper understanding of how each EMIP affects rumen dynamics is necessary before its inclusion. The CNCPS does not attempt to reproduce rumen biology in full; rather, it relies on consistent and biologically meaningful parameters to generate predictions. EMIPs may affect these parameters in ways that are currently unknown, and as such, must be explicitly captured prior to implementation into the CNCPS.

As unique enteric methane-reducing ingredients and additives are developed, solution providers face a steep learning curve regarding data needs, study design, and trial protocols required to support model integration. Data needed goes beyond basic compositional inputs, instead requiring detailed characterization of nutrient dynamics,

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rumen behavior, and associated animal responses. In many cases, successful integration will likely require further rumen submodel development and the addition of decision trees by the CNCPS development team to represent the biological influences of EMIPs with different modes of action.

Implementation of EMIPs into CNCPS takes time. Methane disrupting ingredients or feed additives can influence the cow beyond methane inhibition and must be studied closely before they are adopted into the CNCPS system. Through rigorous testing, producers can gain trust that new products will not hurt the bottom line of the farm or negatively impact cow health.

This document aims to establish standardized research guidelines for incorporating enteric methane-reducing ingredients and additives into the CNCPS to streamline this process. As an outcome, solution providers can approach studies with more cohesive, efficient, and robust research plans. The studies hereafter described should proceed with the recommended dosage determined by solution providers through their own titration studies. The specific data generated, and the methodologies used to obtain them, are critical for the successful incorporation of EMIPs into the CNCPS.

CNCPS Incorporation Criteria

For EMIPs to be incorporated into the CNCPS, the feed ingredient or commercially available feed additive must be fully characterized, and several animal studies must be performed to quantify their impact on rumen metabolism and function. Additional studies that should be conducted are also included and indicated with an *.

- Characterization of feed ingredient or commercially available feed additive
- Animal studies
    - Degradation kinetics
    - Omasal flow
    - Total tract digestibility
    - Methane and performance
    - Energetics and partitioning*

Companies developing feed additives targeted at reducing enteric methane production must complete all studies listed above to comply with the CNCPS data requirements. They *may* also include a study on energy partitioning, which will likely be mandatory in later iterations of the CNCPS with further submodel development. Ingredients that have already

been integrated into the CNCPS library and have already undergone the necessary product characterization must also complete the outlined animal studies or provide the necessary data if such studies have been completed.

A wide range of inputs is needed to properly understand how each EMIP metabolizes in the rumen and the full effects it may have on the cow, including methane reduction. The list of data points provided below is required for the CNCPS to properly estimate the reduced emissions and other metabolic outcomes. A pathway towards EMIP integration is shown below.

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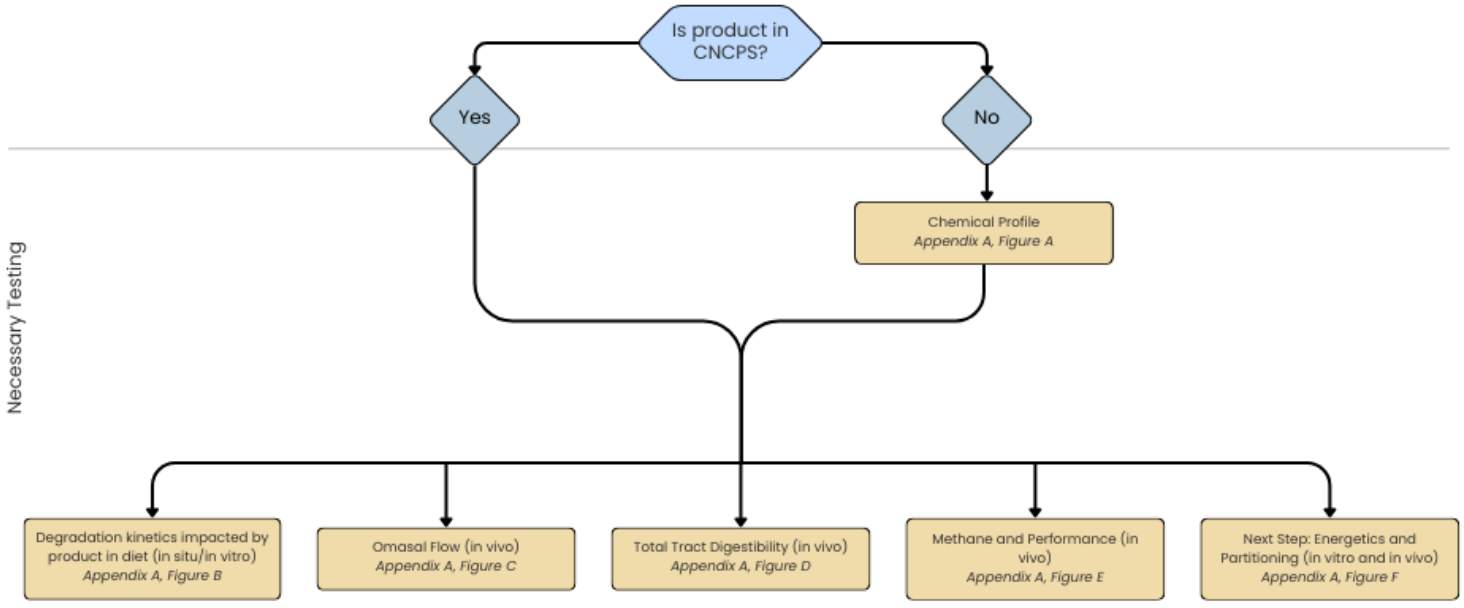
The CNCPS EMIP Integration



*If tests have already been completed outside of CNCPS, duplicate testing may not be necessary.

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Characterization

Characterization of the ingredient or feed additive is the most accessible data to acquire because it does not require the conduction of a feeding trial. Instead, solution providers can send a representative sample of their EMIP to commercial feed laboratories that have standardized methods to characterize livestock ingredients. The composition and chemical makeup of the EMIP itself must be analyzed to the same degree as a standard livestock feed ingredient. This is the baseline requirement for any ingredient to be included in the CNCPS library.

Why the CNCPS needs it: Characterization through wet chemistry analysis (WCA) and digestibility ass

ays will establish the baseline characteristics of the ingredient or additive itself and allow the model to infer to what extent the EMIP breaks down under rumen-like conditions.

Methodology reference(s):

Higgs et al. (2015)	*Updating the Cornell Net Carbohydrate and Protein System feed library and analyzing model sensitivity to feed inputs*
Dineen et al. (2021a)	*Characterization of the nutritive value of perennial ryegrass (Lolium perenne L.) dominated pastures using updated chemical methods with application for the Cornell Net Carbohydrate and Protein System*

Key characteristics:

- A high-quality commercial feed laboratory *must* be utilized for characterization of the EMIP
- Wet chemistry *must* be used [not Near InfraRed (NIR) spectroscopy] for chemical profile analysis
- EMIP developers *should* already have standardized production procedures to create and distribute homogenous products, pending the nature of the product

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Approach	Analyses	Data Needed

Send representative samples of EMIP to a high-quality commercial feed laboratory (examples include Cumberland Valley Analytical Services, Dairyland Laboratories, Inc., etc.)	• Wet chemistry analysis (WCA) for chemical profile	• Dry matter (DM)
		  ○ Organic Matter (OM)
		  ○ Ash
		• Neutral detergent fiber analyzed with alpha-amylase, sodium sulfite, and ash corrected (aNDFom; 12, 30, 120, and 240h sampling time points), acid detergent fiber (ADF), soluble fiber, lignin, sugar, starch, water-soluble carbohydrates
		• Crude protein (CP) or nitrogen, soluble protein, ammonia, non-protein nitrogen, aNDFom insoluble protein, ADF insoluble protein, undegradable nitrogen, amino acids
		• Volatile fatty acids, lactic acid, and other organic acids
		• Ether extract and fatty acids
		• Vitamins

	• *In vitro* digestibility	• Digestibility of protein, NDF, and starch

Animal Studies

Characterization of the EMIP chemical profile will provide valuable insights into how it will be generally processed by the rumen. However, more in-depth animal (*in situ*/*in vivo*) studies where the EMIP is included in the diet for extended periods (with a minimum of 90 days adaptation) are required to understand the entire impact of the EMIP on animal performance.

Completing high-quality animal research is a significant, but invaluable financial investment. Proper planning and execution can allow for multiple categories of data to be collected within a single study. Being efficient with study design is not only in a solution provider's financial interest but it is also aligned with the 3Rs of animal research: **R**educing animal use, **R**efining methods, and **R**eplacing unnecessary procedures wherever possible (Festing and Wilkinson, 2007).

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Research trial expenses will vary by contract research organization (CRO) or university, but some estimated costs to consider are animal care, facility and equipment fees, sampling materials and laboratory analyses, skilled personnel and labor, and possible indirect or overhead costs. A trial scenario of 24 cows (12 control and 12 treatment) for 104 days (90 days for adaptation to diet, 14 days of measurement) is presented with a range of expected costs in Table 1.

Table 1. Example price points of the critical resources required for a dairy nutrition study

Cost category	Estimated range	104-day with 24 cows	What it covers
Animal Care	$10-20 / cow / day	$2,4960 - 49,920	Feed, bedding, daily and routine health care
Facility & Equipment	$5-25 / cow / day	$12,480 – 62,400	Barn fees for use of stalls, parlors, and data systems. Upper end includes operating costs of gas equipment
Sampling & Laboratory Analyses	$800-2,500 / cow	$19,200 - 60,000	Feed, milk, and other biological samples for analysis
Personnel/ Labor	$500-1,200 / day	$52,000 – 124,800	Feeding, sample collection, study management, lab prep, and data entry
Total (before overhead)	**$108,640 – 297,120**		
Indirect costs/ Overhead	Up to 40-60%	If conducted as a product test at Cornell University, the overhead is 37% and this must have approval from the college before initiation. If conducted as a contract, the overhead is 57% and this is developed with the Office of	

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		Sponsored Programs at Cornell in the College of Agriculture and Life Sciences. *Intensity level is determined by type, quantity, and frequency of data collection*.

The more sampling that can be layered within a study, the more it can lessen the burden of future animal trials. Suggested approaches for layered study design will follow the outline of individual studies provided herein.

Key characteristics of all animal studies to be considered:

- Animals *must* be adapted to the same basal diet and experimental treatment for 90 days before study measurements commence
- Latin squares *must* not be deployed as an experimental design
- A control group *must* be included in the experimental design
- Diet and individual ingredients *must* be collected on a weekly basis for WCA; individual ingredients *must* be collected at least two times within the week and composited weekly. For forage ingredients, it is recommended at least three times a week.
- Dry matter of the diet *must* be determined on a weekly basis, and it *should* include at least two samples within the weekly cadence of sampling
- Dry matter of the feed refusals *must* be determined on a weekly basis, and it *should* include at least two samples within the weekly cadence of sampling
- Multiple dietary contexts for inclusion of the ingredient or additive *must* be evaluated, as relevant to the EMIP and its mode of action. These contexts may include, but are not limited to, high- vs low-forage diets, diets differing in NDF disappearance, diets differing in rumen nitrogen balance, and variation in metabolizable energy and metabolizable protein supply. Consultation with the CNCPS development team is crucial prior to study implementation to ensure the appropriate dietary contexts are evaluated.

1. Degradation Kinetics Study

The purpose of this study is to determine the degradation rates (kd) of degradable fractions in the rumen once adapted to the EMIP.

Why the CNCPS needs it: If the presence of an EMIP in a diet changes the ruminal degradation of dietary substrates, updated kd allow the model to correctly predict the extent of rumen degradation and subsequent VFA production and microbial substrate supply.

While an *in vitro* approach is preferred, data collection can be achieved through *in situ* or *in vitro* approaches depending on resource and logistical limitations.

Option A: In vitro approach

Methodology reference(s):

Goering and Van Soest, (1970)	Forage fiber analysis
Raffrenato et al., (2018)	Development of an in vitro method to determine rumen undigested aNDFom for use in feed evaluation
Ross et al. (2013)	Development of an in vitro intestinal digestibility assay for ruminant feeds

Approach (Option A)	Data needed
Conduct an animal study and deploy *in vitro* methods. Collect the rumen fluid from EMIP-adapted animals to perform analyses within an *in vitro* system.	Disappearance % of • Protein • NDF • Starch

Key characteristics *in vitro* degradation study:

- Daisy Fermenters *should* not be used
- Rumen fluid *should* be collected from at least 3 donor animals
- All samples *must* be run in duplicate

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- If used, pore size of samples bags *must* not be smaller than 1.5µ or larger than 25µ
- Ingredients *must* be observed independently
- Forages, high-nitrogen, and high-NFC feeds *must* be evaluated
- Sampling *should* occur over at least eight points and will vary depending on the metric of interest
    - Protein: (preferred timepoints of 0, 6, 10, 12, 18, 24, 30, and 36 h, however can vary depending on the ingredient or feed additive)
    - Neutral Detergent Fiber:(minimum timepoints of 12, 30, 120, and 240 h for forages and 12,72, and 120 h for non-forages)
    - Starch:(preferred timepoints of 0, 3, 6, 9, 12, 18, 24, and 30 h)

Option B: *In situ* approach

Methodology reference(s):

Norris et al. (2019)	*Assessment of in situ techniques to determine indigestible components in the feed and feces of cattle receiving supplemental condensed tannins*

Approach (Option B)	Data analyses
Conduct an animal study and deploy *in situ* methods. With cannulated animals adapted to EMIP diet, analyses can be performed within the rumen environment.	Disappearance % of • Protein • NDF • Starch

Key characteristics of *in situ* degradation study:

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- Animals *must* be cannulated
- Experimental design *must* include at least 3-4 animals and Latin square designs are discouraged due to carryover effect
- All samples *must* be run in duplicate
- Pore size of sample bags *must* not be smaller than 1.5µ or larger than 25µ
- Ingredients *must* be observed independently
- Forages, high-nitrogen feeds, and high-NFC feeds *must* be evaluated
- Sampling for each compound *should* occur over several time points and will vary depending on the metric of interest
    - Protein: Preferred timepoints of 0, 6, 10, 12, 18, 24, 30, and 36 h, however, can vary depending on the ingredient or feed additive
    - Neutral detergent fiber: Minimum timepoints of 12, 30, 120, and 240 h for forages and 12,72, and 120 h for non-forages
    - Starch: Preferred timepoints of 0, 3, 6, 9, 12, 18, 24, and 30 h

2. Omasal Flow Study

An omasal flow study quantifies the true ruminal outflow of digesta and microbes, providing direct measurements of nutrient passage from the rumen to the intestine.

Why the CNCPS needs it: The CNCPS relies on accurate estimates of nutrient and microbial flow to the intestine. Omasal flow measurements provide a necessary reference for adjusting model parameters governing rumen outflow, ensuring that predicted metabolizable energy and metabolizable protein supply remain biologically consistent when EMIPs are incorporated into diets.

Methodology reference(s):

Reynal et al. (2007)	*Omasal Flow of Soluble Proteins, Peptides, and Free Amino Acids in Dairy Cows Fed Diets Supplemented with Proteins of Varying Ruminal Degradabilities*

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Fessenden et al. (2019)	*Rumen digestion kinetics, microbial yield, and omasal flows of nonmicrobial, bacterial, and protozoal amino acids in lactating dairy cattle fed fermentation by-products or urea as a soluble nitrogen source*
Dineen et al. (2021b)	*Microbial composition and omasal flows of bacterial, protozoal, and nonmicrobial amino acids in lactating dairy cows fed fresh perennial ryegrass (Lolium perenne L.) not supplemented or supplemented with rolled barley*
Denton et al. (2015)	*Accumulation of Reserve Carbohydrate by Rumen Protozoa and Bacteria in Competition for Glucose*

Approach	Data analyses
Conduct an animal (*in vivo*) study using cannulated cattle, dose multiple digesta markers, and sample rumen and omasal digesta to quantify flows needed to parameterize digestion and passage dynamics.	• Solid passage rate (kp) • Liquid kp • Microbial N flow and pool size • CHO and N flow and rumen pool size

Key characteristics of omasal flow study:

- Animals *must* be cannulated
- Experimental design *must* include at least 6 animals and Latin square designs are discouraged due to carryover effect
- Diurnal variation *must* be captured through a minimum of 12 samples collected over no longer than 72 h, representing a 24-h period
- Rumen evacuations to weigh total rumen content *must* be part of the study design
- Parallel samples of rumen fluid *must* be collected alongside omasal samples

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- Protozoal flocculation *must* be performed as outlined in Denton et al. (2015) and protozoa must not be placed in a high-sodium buffer
- Omasal markers may include uNDFom, rare-earth for solids and co-EDTA for liquid

3. Total Tract Digestibility Study

Total tract digestibility determines overall nutrient disappearance throughout the gastrointestinal tract.

Why the CNCPS needs it: Determines whether digestibility coefficients need adjustment when the EMIP is included in diets.

Methodology reference(s):

Dineen et al. (2020)	*Rumen metabolism, omasal flow of nutrients, and microbial dynamics in lactating dairy cows fed fresh perennial ryegrass (Lolium perenne L.) not supplemented or supplemented with rolled barley grain*

Approach	Data analyses
Conduct an animal (*in vivo*) study where cattle receive the test diet (via spot or total collection) and compare nutrient or marker-corrected outputs to dietary intake	WCA on diet, orts, and manure to determine total tract digestibility of: • DM • OM • Protein • NDF • Starch

Table 2. An example 3-day fecal sampling scheme example to satisfy minimum requirements

Day	Time For Sample Collection

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One	00:00, 02:00, 04:00, 06:00
Two	08:00, 10:00, 12:00, 14:00
Three	16:00, 18:00, 20:00, 22:00

Key characteristics of total tract digestibility study:

- Experimental design *must* include at least 6 animals and Latin square designs are discouraged due to carryover effect
- The sampling *must* occur at least 12 times over 72 hours to capture every other hour in the day. Samples can be blocked into 3 blocks of 6-8 h (Table 2) or samples can be taken every 6-8 h to achieve the required number of samples.
- uNDF *may* be used as a marker
- Samples of respective individual orts (feed refusals) *must* be collected

4. Methane and Performance Study (or Metanalysis)

A methane and performance study generates paired measurements of enteric methane emissions and animal productivity under controlled dietary conditions. These data quantify the magnitude and consistency of methane suppression associated with an EMIP while simultaneously documenting any changes in animal performance. Data can be collected either by conducting animal studies with multiple dietary contexts (see above) utilizing continuous methane measurements (Option A) or compiling acceptable spot sampling data collected by GreenFeed for a metanalytic approach (Option B). Option B is only acceptable as an approach if the estimated methane reduction factor of the ingredient or additive is greater than (>) 20%. Submodels may be developed in the future that are mechanism-specific to estimate methane reduction.

Why the CNCPS needs it: The CNCPS currently predicts enteric methane emissions as a function of metabolizable energy intake and dietary carbohydrate characteristics. Methane measurements are therefore required to validate model performance when EMIPs are included in the diet and to determine whether the observed reductions in methane are consistent with the predicted changes in the underlying drivers. These data provide a critical reference point for evaluating whether existing model structures adequately

capture EMIP effects or whether additional development of the rumen submodel is required to address distinct modes of action. Without empirical methane measurements linked to performance outcomes, the CNCPS cannot confidently adjust its predictions or ensure that modeled methane reductions remain biologically consistent with energy partitioning and animal productivity.

Option A (Preferred): Continuous gas measurement study

Methodology reference(s):

Respiration chamber (RC)	Machado et al. (2016)	Technical note: A facility for respiration measurements in cattle
Portable accumulation chamber (PAC)	Morris and Kononoff (2021)	*Derivation of the maintenance energy requirements and efficiency of metabolizable energy utilization for dry and lactating Jersey cows*
	Freetly et al. (2006)	*Partitioning of energy during lactation of primiparous beef cows*

Approach	Data Analyses
Conduct an animal (*in vivo*) study measuring continuous gas emissions from cattle receiving the test diet using a standardized system (respiration chambers or portable accumulation chambers) while recording performance parameters	• DMI and water intake [A] • MY, milk protein, milk fat, lactose, ECM yield, milk fatty acids, MUN [B] • Gas (CH_4, CO_2, H_2; grams per day) [C] • Body weight, BCS, ADG, FCE [D] • Environmental characteristics [E]

A: DMI = dry matter intake
B: MY = milk yield, ECM = energy corrected milk (ECM = (milk yield × 0.327) + (fat yield × 12.95) + (protein yield × 7.65); Tyrell and Reid, 1965), MUN = milk urea nitrogen
C: CH_4 = methane, CO_2 = carbon dioxide, H_2 = hydrogen
D: BCS = body condition score, ADG = average daily gain, FCE = feed conversion efficiency
E: Temperature, humidity, airflow

Key characteristics of methane and performance study (continuous gas):

- Sample size *must* be determined by a power analysis using anticipated differences in methane output
- Methane *must* be continuously measured using either 1) respiration chambers (RC), or 2) portable accumulation chambers (PAC)
- Animals *must* be acclimated to RC or PAC before measurements. A minimum of 48 h is recommended; however, animals *must* demonstrate a stable DMI and a lack of distress through rumination and laying behaviors which many require longer adaptation periods
- This option *must* be pursued if the anticipated absolute methane reductions are less than (<) 20%

Option B: Metanalyses of spot samples

Spot sampling through GreenFeed can be acceptable as methodology under certain specifications. Only EMIPs that have an anticipated reduction in methane emissions greater than 20% can take this approach. Data will need to be compiled over several studies.

Methodology reference(s):

Hristov et al. (2015)	The use of an automated system (GreenFeed) to monitor enteric methane and carbon dioxide emissions from ruminant animals.

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French et al. (2025)	Experimental and experiential recommendations for using the GreenFeed systems to measure gas flux in grazing and confined cattle

Key characteristics of methane and performance study (spot gas samples):

- There is no minimum number of treatment means that must be included in the metanalysis of spot sampling data. However, the compiled dataset *must* provide sufficient confidence, precision, and consistency in the estimated methane reduction. Specifically, the pooled effect should be statistically different from zero, the uncertainty around the estimate should be small relative to the magnitude of the effect, and the direction of response should be consistent across independent studies.
- Sample size per study *must* be determined by a power analysis using anticipated differences in methane output
- Spot sampling *must* be collected by flux technology that can collect data on an individual animal basis (i.e. GreenFeed)
- Sampling schedule must reflect a 24-hour timescale either by allowing free access to quantification technology (freestall) or designing an artificial sampling schedule (tiestall; see Hristov et al. 2015)
- A minimum of 40 samples of ≥ 2 min in duration per animal, or 30 samples of ≥ 3 min in duration per animal are required

Approach	Data needed
Compile treatment averages or means from several qualifying *in vivo* studies where spot sampling methods were deployed	• DMI[A] • MY, milk protein, milk fat, lactose, ECM yield, milk fatty acids, MUN[B] • Gas (CH_4, CO_2, H_2; grams per day) [C] • Body weight, BCS, ADG, FCE[D]

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	• Environmental characteristics [E]
A: DMI = dry matter intake B: MY = milk yield, ECM = energy corrected milk (ECM = (milk yield × 0.327) + (fat yield × 12.95) + (protein yield × 7.65); Tyrell and Reid, 1965), MUN = milk urea nitrogen C: CH_4 = methane, CO_2 = carbon dioxide, H_2 = hydrogen D: BCS = body condition score, ADG = average daily gain, FEC = feed conversion efficiency E: Temperature, humidity, airflow	

Table 4. Spot gas sampling requirements

Input	Requirement
Number of total samples	40 samples of ≥ 2 min in duration per animal, or 30 samples of ≥ 3
Days of sampling	10-14; Determined primarily to reach minimum samples
Time buckets	6 or 8 hours
Minimum daily samples per animal	3

5. Energy Partitioning Study*

An energy partitioning study provides detailed accounting of how dietary energy is allocated among maintenance, productive functions, and losses. The resulting data quantify both energy retention and energy dissipation pathways, allowing a clear assessment of how EMIPs alter energetics at the whole-animal level.

Why the CNCPS needs it: The CNCPS predicts animal performance and environmental outputs by tracking energy flows through biologically defined pathways. Energy partitioning data are therefore essential to further validate existing model predictions and to identify where current representations may be insufficient when EMIPs are included in diets.

Methodology reference(s):

Sutton et al. (2003)	Rates of production of acetate, propionate, and butyrate in the rumen of lactating dairy cows given normal and low-roughage diets

Key characteristics of energy partitioning study:

- Sample size *must* be determined by a power analysis using anticipated differences in methane output
- Methane *must* be continuously measured using respiration chambers (See Key characteristics of methane and performance study (continuous gas), above)
- Animals must be acclimated to RC or PAC before measurements. A minimum of 48 h is recommended; however, animals must demonstrate a stable DMI and a lack of distress through rumination and laying behaviors which many require longer adaptation periods

Approach	Data Analyses
Conduct an animal (*in vivo*) study measuring continuous gas emissions from cattle receiving the test diet using a standardized system while recording rumen fermentation, energy losses, and performance parameters	• DMI and water intake[A] • MY, milk protein, milk fat, lactose, ECM yield, milk fatty acids, MUN[B] • Gas (CH_4, CO_2, H_2; grams per day)[C] • Body weight, BCS, ADG, FCE [D] • Rumen fermentation profile (VFAs) and pH [E] • Fecal energy and nitrogen (MJ or Mcal) • Urinary energy and nitrogen (MJ or Mcal) • Environmental characteristics (temperature, humidity, airflow)

A: DMI = dry matter intake
B: MY = milk yield, ECM = energy corrected milk (ECM = (milk yield × 0.327) + (fat yield × 12.95) + (protein yield × 7.65); Tyrell and Reid, 1965), MUN = milk urea nitrogen
C: CH_4 = methane, CO_2 = carbon dioxide, H_2 = hydrogen
D: BCS = body condition score, ADG = average daily gain, FEC = feed conversion efficiency

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Layering of Studies

Studies can be layered within each other for efficiency. For example, omasal flow and total tract digestibility studies can be tested together because they require relatively the same population size if the animals are cannulated. Methane and performance study and energy partitioning study can also be tested together due to the overlap in data collection and similar population sizes required.

It is possible to layer all the studies together and maintain sampling for the degradation study, total tract digestibility study, and omasal flow study, from small subgroups within either the methane and performance or energy partitioning studies, since those studies will require greater population sizes.

Next Steps: CNCPS Development Team

Once all the necessary tests are completed, information can be forwarded to the CNCPS team at (vanutritionlab@cornell.edu). The CNCPS team will be in contact with EMIP solution providers once data is reviewed, and they will either communicate approval and next steps or areas of improvement to ensure data quality standards are upheld.

The submission fee to the CNCPS is $2,500 per ingredient for curation into the reference library for distribution to all commercial software companies. This cost considers the time and effort put forth by the CNCPS development team to incorporate EMIPs into the feed library.

Solution providers should not anticipate rapid implementation of their products into the CNCPS following the provision of the required data. EMIPs introduce complex shifts in rumen metabolism, including effects on hydrogen flux that further stress carbohydrate degradation kinetics, microbial communities and proliferation, and other fermentation dynamics that all interact across the submodels within the CNCPS. These changes directly affect the supply of metabolizable energy and metabolizable protein, and in turn, influence animal efficiency and productivity. Therefore, extensive validation of the model following submodel adjustments must occur. The estimated cost of this modeling exercise will be disclosed once the group understands the complexity of the task and it is **strongly recommended to engage the group prior to committing to all the chemistry and**

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research to ensure that all conditions for model enhancement have been met prior to this step.

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Appendix A: Study Integration Pathways

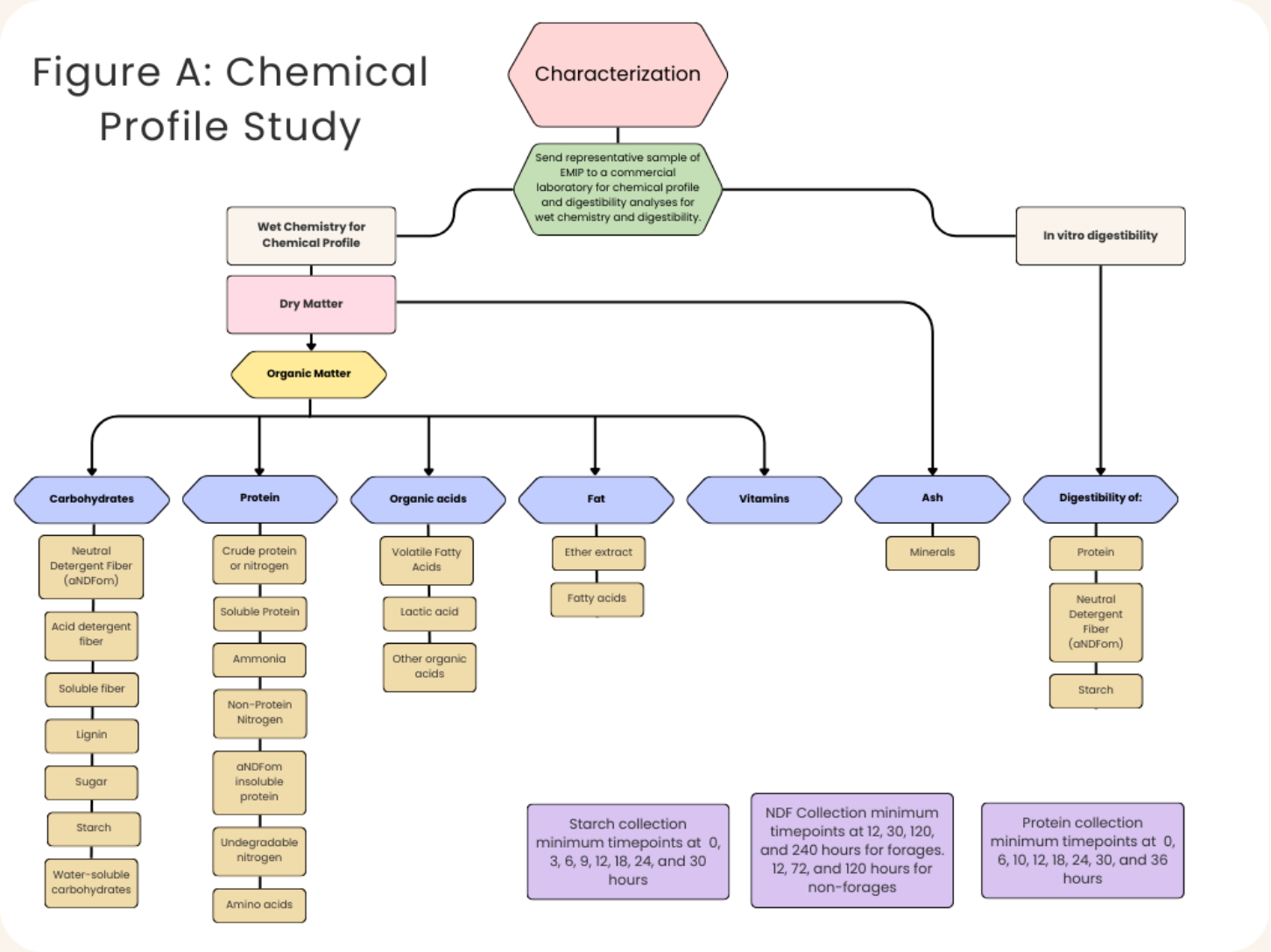

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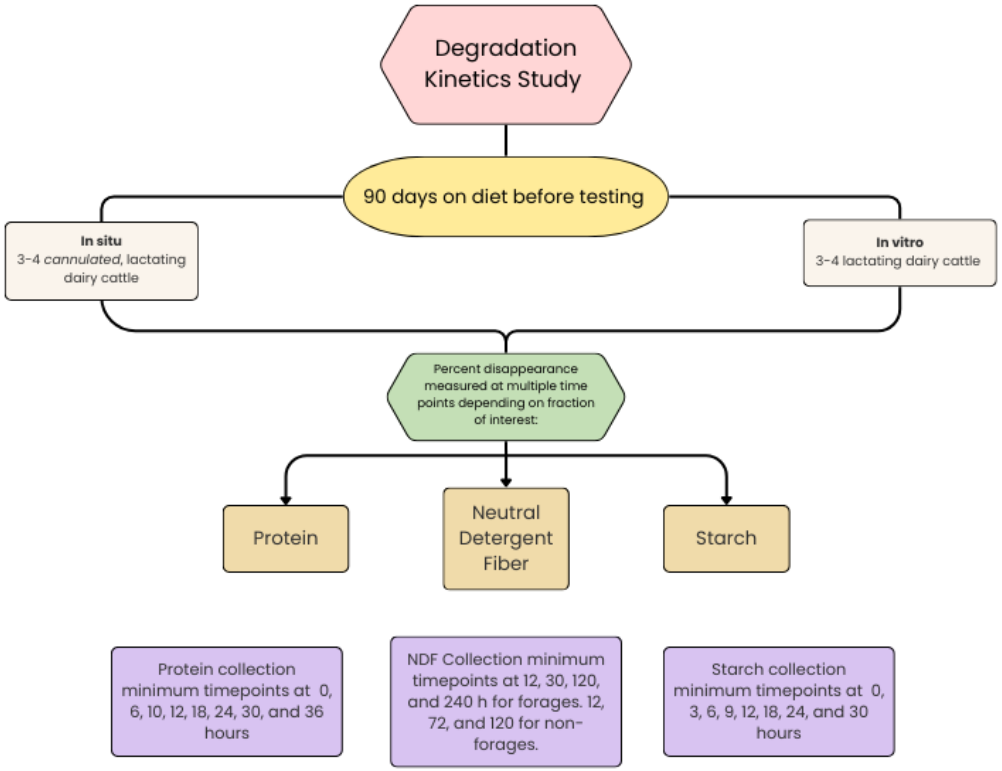
Figure B: Degradation Kinetics Study



*Can be done alongside omasal flow and total tract digestibility if using in vitro method.

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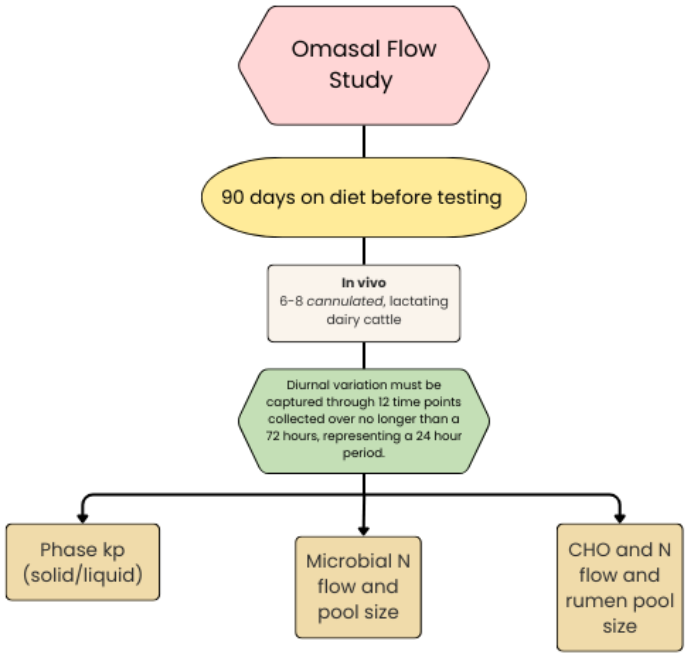
Figure C: Omasal Flow Study



*Can be done alongside degradation kinetics if degradation kinetics uses in vitro. Data for omasal flow can be collected alongside total tract digestibility.

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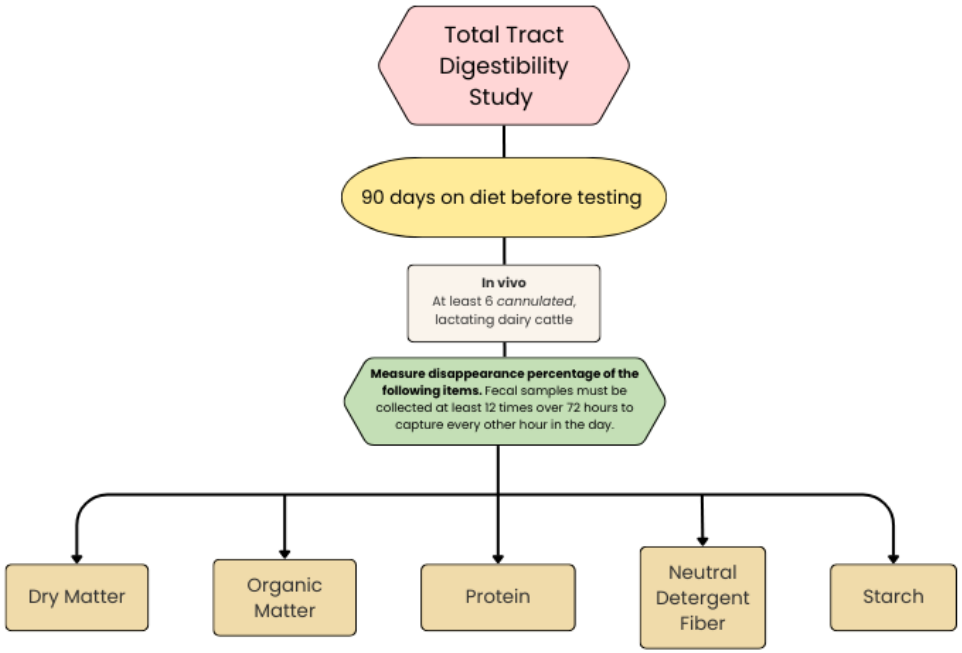
Figure D: Total Tract Digestibility Study



*Can be done alongside degradation kinetics if degradation kinetics uses in vitro. Data for total tract digestibility can be collected alongside omasal flow.

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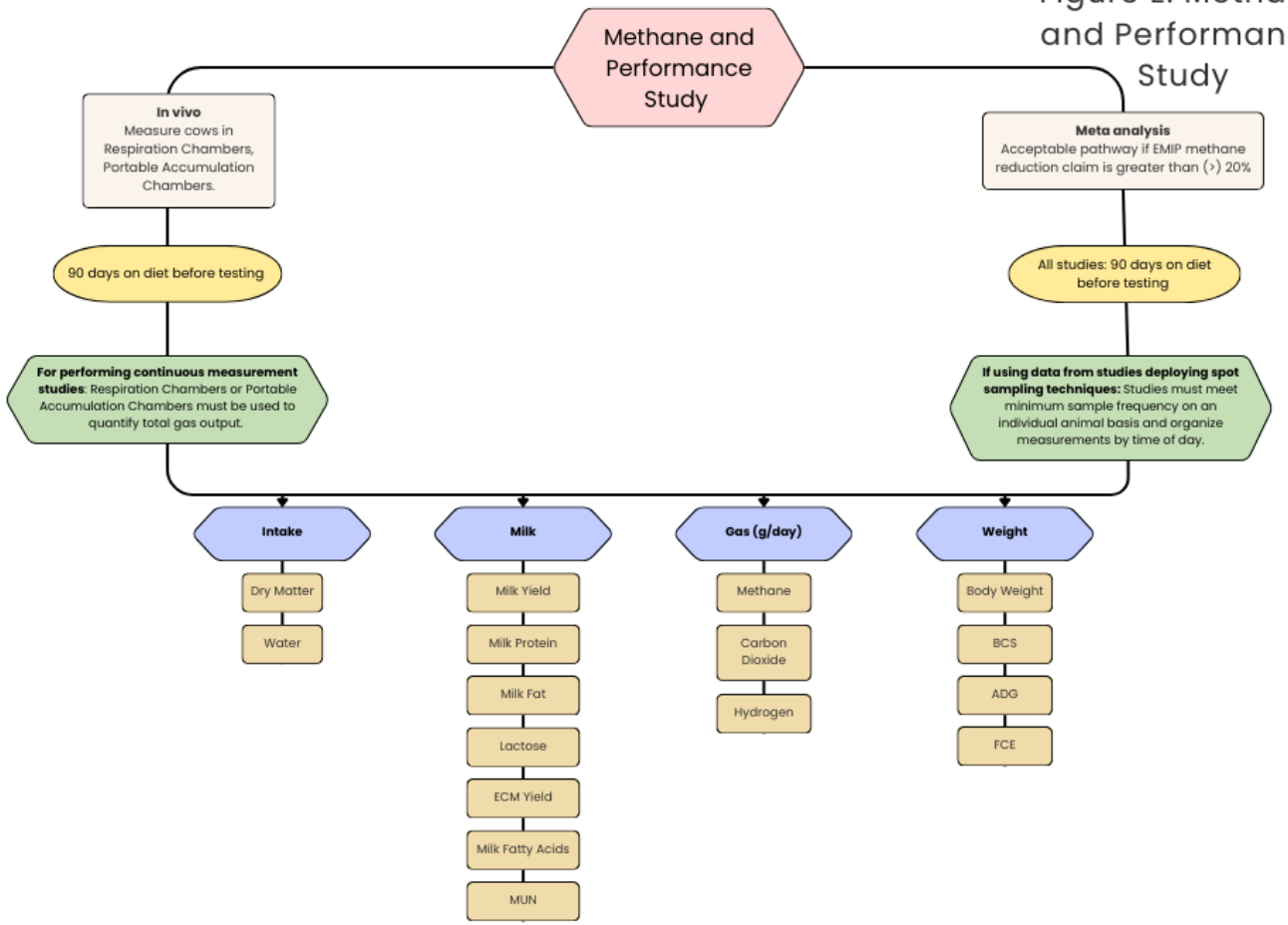
Figure E: Methane and Performance Study



*Data for methane and performance can be collected alongside energetics and partitioning if using in vivo.

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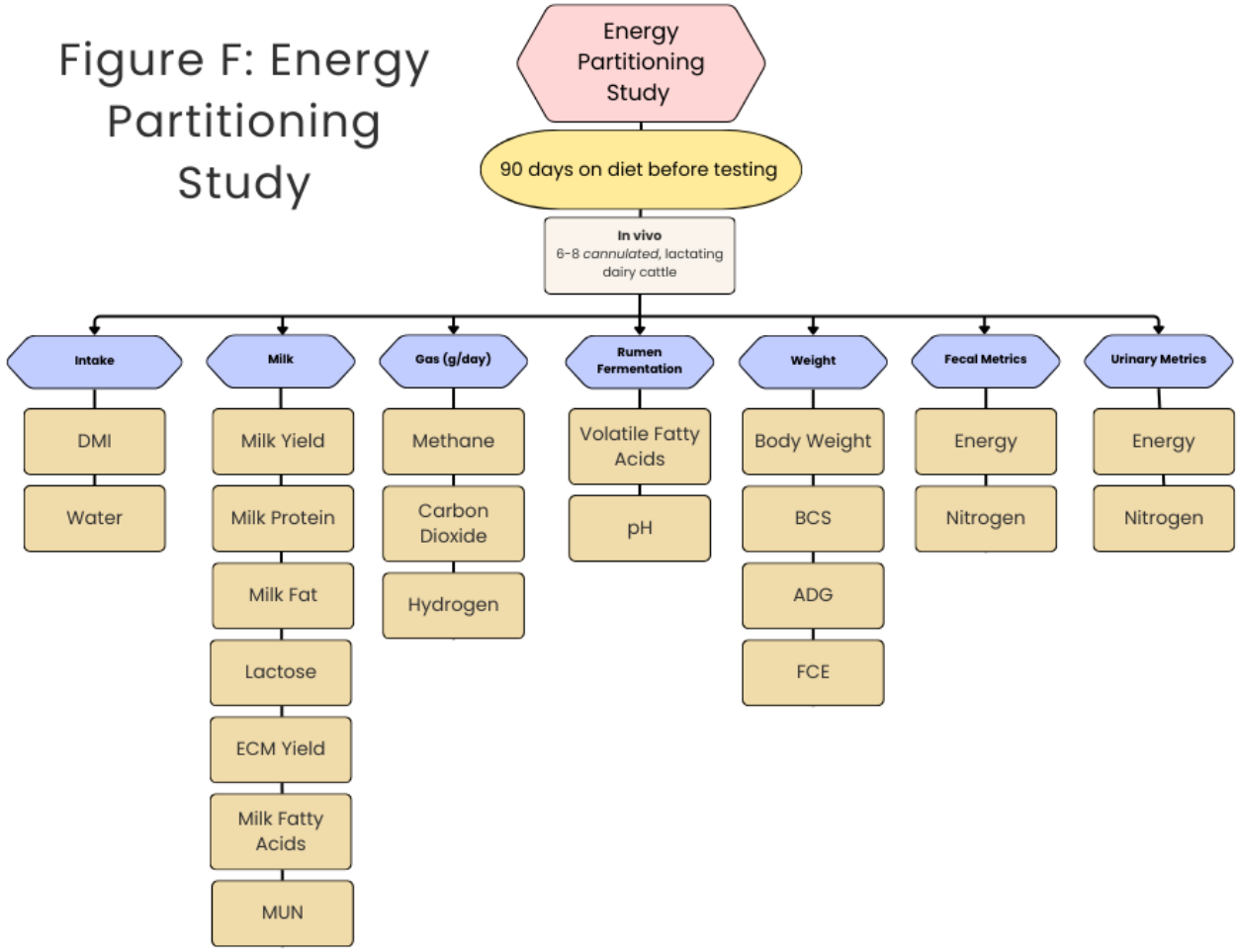
Figure F: Energy Partitioning Study



*Data for energetics and partitioning can be collected to encapsulate methane and performance.

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Appendix B: Sources

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