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Life Fiber TECHNOLOGY™

DECIPHERING THE DIETARY FIBER MESSAGE

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What if you are not just what you eat, but also what your microbes eat?

— John Cryan

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Message in a Bottle

Our knowledge base on dietary fiber has been expanding briskly during the past two decades; this information, and how to apply it, is progressing so rapidly that it has become a time-consuming challenge to keep up with the discoveries. As a reminder, a few years ago fiber was considered inert and an indication of poor quality, whereas today fiber is one of the most exciting areas of study in nutrition with enormous possibilities. In addition, we are entering an era where the focus will be to nourish the microbiome to promote health and well-being.

Dietary fiber is complex; it is a short message, but is clear to animal nutritionists. The key to unraveling this complexity is interpreting the analytical data and the biological response that fibrous ingredients will elicit. Dietary fibers can be viewed as beneficial or detrimental based on the context of the total diet. When dietary fibers are poorly fermented, they still promote laxation, epithelial cell regeneration, and favorable stool quality. When dietary fibers are rapidly fermented, they provide nourishment for the intestinal bacteria, which in turn produce beneficial metabolites that promote animal health. In contrast, excessive fermentable dietary fiber can result in osmotic imbalances and other intestinal disorders. What are some of the new ways of viewing and characterizing fiber and how can they help us construct improved diets?

Capturing Dietary Fiber

According to the American Association of Cereal Chemists (AACC) "Dietary fiber is the edible portions of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine and are either completely or partially fermented in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin and associated plant substances." (AACC, 2001). However, the analytical methods available do not always align with this definition.

Dating back to the Weende system of proximate analysis created in the 1860s, the term crude fiber was developed to estimate the indigestible fractions of a feedstuff (AAFCO, 2017). This method to classify dietary fibers is as the name suggests, crude, and has been found to have very little useful information in modern diet formulation for simple stomached animals. This methodology accounts for most of the cellulose, but only a portion of the hemicellulose and lignin, resulting in substantial underestimation of the true fiber content. Furthermore, crude fiber does not account for the soluble fiber in an ingredient and provides little knowledge about the functionality and fermentability. Unfortunately, this methodology and label guarantee is still used to this day.

Numerous other methodologies are also available with detergent methods and total dietary fiber methods being the most prevalent. The detergent methods include neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL). These methodologies allow one to identify the insoluble hemicellulose, cellulose, and lignin, respectively, within a sample. The total dietary fiber (TDF) methodology accounts for a broader array of fiber types that can be partitioned into insoluble dietary fiber (IDF) and soluble dietary fiber (SDF). In combination with detergent analyses, one can develop an understanding of the chemical composition and functionality of a dietary fiber source.

Splitting the Difference

In further detail, IDF includes fiber types, such as lignin, cellulose and select hemicelluloses, that vary in microbial fermentability and importance to gastrointestinal development, intestinal motility and stool firmness. These fiber types nourish the microbiota, as well as synchronize the movement of digesta with internal cues. They also assist with the osmotic balance inside the intestine, which helps with stool quality. On the contrary, IDF increases stool volume, which can be unfavorable to the perception of diet quality for pet owners and livestock producers. There is an optimal quantity of IDF required to promote laxation, but also prevent excessive fecal volume.

Soluble dietary fiber includes select hemicelluloses, oligosaccharides, fructans, beta-glucans, and pectins. These fiber types vary in fermentability and most serve as important substrates for gut bacteria. Each varies in the rate and location of fermentation and the microbial metabolites produced. In addition, these fiber fractions include beta-glucans and prebiotics, famously known for good intestinal health. Soluble fibers are thought to provide many of the health benefits associated with dietary fiber.

Detergent fiber and total dietary fiber methodologies are much more descriptive than earlier fiber

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classifications, but it is important to note that these are only values based on the analytical methodology used to measure it. It does not explain the physiological effects or the complexity of the fiber substrate, since sources of dietary fiber are heterogeneous and their chemical composition does not tell the whole story. Therefore, formulating on analytical values alone can be misleading and harmful to animal health and performance.

The chemical structure of an ingredient influences factors such as fermentation and microbial growth rate, intestinal viscosity, and microbial diversity. In addition, chemical structure influences gut functionality and how information is transferred to the microbiota and host. An ingredient predominantly comprised of mostly insoluble fiber can be partially fermentable (e.g., wheat bran) and ingredients comprised of mostly soluble fiber are not always fermentable (e.g., psyllium; Bourquin et al., 1992; Campbell and Fahey, 1997), but both influence information transfer during digestion and fermentation. Understanding the fermentation rate and degree that a fiber source will be degraded is important for determining the physiological effect and location of that effect in the gut. Many ingredients, such as pectins and psyllium, can greatly increase the viscosity of the digesta, which can alter digesta passage rate, nutrient absorption, and intestinal fermentation. The chemical structure of a fiber source, such as branching and degree of polymerization (DP), affect the microbial accessibility and rate at which a fermentable carbohydrate is degraded. As DP increases, transit rate and rate of fermentability decrease, causing the fiber to be fermented more distally in the large intestine (Rumessen and Gudmand-Høyer, 1998; Stewart et al., 2008). This in turn affects microbial activity because some bacterial species only possess the enzymes to ferment longer chain oligosaccharides. In addition, one cannot think about dietary fiber sources in isolation, but the entire diet matrix and form must be considered, since the cecal and large intestinal microbiome is influenced by all undigested compounds in the digesta and not just dietary fibers. The complexity of dietary fiber, the microbiome, and the flow of information during fermentation is staggering.

Fermentable Load

John Cryan said, "we [animals] are not just what we eat, but what our microbes eat," and we propose the term "fermentable load" to describe the total amount of substrate that will be available for microbial fermentation in the cecum and large intestine of monogastrics. The fermentable load is predominately comprised of undigested carbohydrates and proteins that escaped hydrolytic digestion in the small intestine. Therefore, fermentable load is the available nutrients for the microbiome in the hindgut.

Dr. Neil Jaworski with Fiber Analyzer

Formulating with the proper amounts and ratios of dietary fibers that are fast and slowly fermented promote carbohydrate fermentation throughout the lower gut. For instance, if undigested proteins dominate the fermentable load, less efficient protein fermentation dominates, resulting in the production of toxic compounds, such as ammonia, biogenic amines, phenols, indoles, and gases, such as methane and hydrogen sulfide, which can lead to increased risk for diarrhea and gastrointestinal upset. Furthermore, gases, phenols, and indoles result in flatulence and stool odor, which affect consumer perception of diet quality (Yao et al., 2015). Promoting carbohydrate fermentation within the fermentable load is particularly important in the distal large intestine, when often carbohydrates have been exhausted and nutrients are limited.

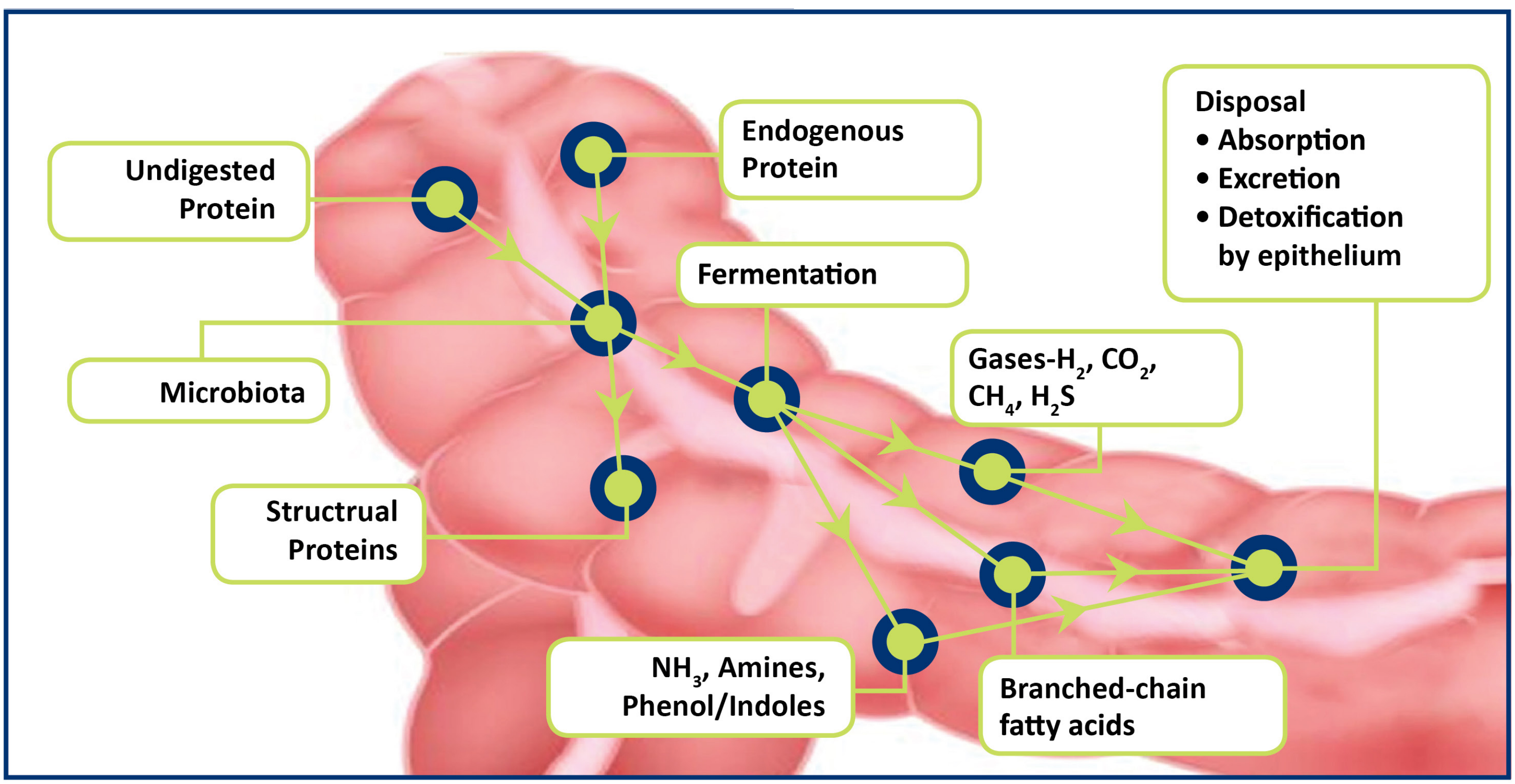
Studies have shown that additional fermentable fibers can repartition nitrogen excretion from urinary nitrogen to fecal microbial protein (Zervas and Zijlstra, 2002). As microbes have additional fermentable fibers available, replication is increased and a greater concentration of nitrogen is assimilated into microbial protein as opposed to being excreted via urine. The fermentable load provides the formulator an estimation of the nutrients available for fermentation by the microbiome to promote carbohydrate fermentation and suppress the fermentation of undigested protein entering the lower gut.

Finally, the fermentable load helps prevent over-feeding the microbes and under-feeding the animal. This leads to reduced animal efficiency because fermentation is not as efficient of an energy source as hydrolytic digestion. For instance, some studies have reported reduced feed efficiency by pigs fed increased inclusion levels of wheat middlings, whereas pigs fed similar levels of distillers dried grains with solubles (DDGS) maintained feed efficiency (Overholt et al., 2016; Salyer et al., 2012). These studies had similar concentrations of dietary fiber in the diets, but lacked an understanding of fermentation rate and fermentable load. Wheat middlings are more fermentable compared with DDGS, and we hypothesize that feed efficiency was reduced because the fermentable load in the wheat middlings diet exceeded the maximum amount of fermentation possible. The age and physiological stage of the animal, as well as the time with which the microbiome has been provided to adapt to a certain fermentable load, will influence the maximum amount of fermentation and, therefore, the fermentable load is a way to gradually increase the capacity of microbial fermentation and production of health providing metabolites over an animal's lifetime.

To summarize: the fermentable load quantifies the total fermentable substrate available to the microbiome in the cecum and large intestine of pets and monogastric farm animals. Certain dietary fiber fractions not only significantly impact this quantity, but add to our nutritional understanding of physiological effects and fermentation in the animal. The fermentable load helps nutritionists balance the double-edged sword of increased diarrhea risk and reduced animal efficiency as total fermentation is increased in an animal.

Nutrient Synchronicity

It is truly amazing how much we know about the ingredients we use to formulate animal diets. Because of the enormous amount of information we have gathered about ingredients, we know far more about diet formulation for specific outcomes in animals and pets than we do for humans. There are several reasons for this disparity, but clearly one of the reasons has



been the ability to correlate and statistically validate this enormous amount of information about ingredients with performance and phenotypic outcomes of farm animals and pets. Maybe it is more amazing that we continue to find more information about ingredients. Dietary fiber with all the chemical and kinetic characterization is a good example of the tremendous amount of data we have amassed and we have coined the term nutrient intelligence to describe this highly complex data set.

Only a few years ago, fiber was considered of little or no nutritional value to simple stomach animals but this has changed rapidly during the past decade. Dietary fibers' relation to animal health is a major research area in humans, farm animals and pets. Most of the research has been on the relationship of the chemically defined components of fiber and little has been done on the fermentation rates of fiber – however, this is changing. Ingredient kinetic information developed by Trouw Nutrition R&D allows us to incorporate time into ingredient characterization that adds a new dimension to linear diet formulation. We have strived to develop ingredient information that is additive in linear modeling but we know that the flow of information through the animal is not linear; the addition of this new technology neutralizes a portion of that variation. Our nutrition models have served us well but incorporating time or kinetics into the models will enable them to be more predictive. Recent research has found in some cases that kinetic information allows for equal or greater predictability than ideal protein; we believe that the impact on productivity and health in young animals will be comparable to what happened to animal productivity when we made the change from using lysine and methionine requirements to ideal protein (Truong et al., 2017; Liu et al., 2018; Moss et al., 2018).

From the beginning, life has been subject to the daily rhythms of light and dark, which has made a significant impact on living systems due to the drive for organisms to remain mirror-like images of the environment. It has become accepted that humans, animals and microbes are metabolically integrated and coordinated by outside cues known as zeitgebers or external time givers, which are necessary components of the environment that synchronize metabolic activities, hormone balance, DNA and cellular repair and other necessary cellular functions. The most important cues, which modify metabolism, are light, temperature and eating times; collectively they synchronize the cyclic behavior of metabolism to a 24-hour day. Nutritional studies in humans and animals have convincingly revealed that when to eat is as equally important as what to eat. Since most all life processes are controlled by oscillating events of which eating time is a major cue to synchronize biochemical activities, it is not a stretch to visualize digestion rates, which are extensions of eating

Deciphering the complexity of dietary fiber begins to transpire when a critical dietary component is taken into account: time.

time, modifying nutrient delivery rates and having an influence on such things as growth rate, energy partitioning, reproduction, and general well-being of the animal. When nutrient flow is synchronized to the diurnal cycle, the fermentable load needs to be factored into this flow as well. The goal is to match the nutrient requirements of the microbial species that are oscillating through the daily cycle (Sinturel et al., 2017; Parker et al., 2019; Uhr et al., 2019) to ensure the appropriate nutrients are delivered at the proper moment in the cycle.

Within the past decade, animals are becoming increasingly viewed as open systems rather than biological machines, which opens the door for understanding how important cyclic exogenous cues are for animal phenotype. Eating times, quantity of food and meal frequency are all associated with animal behavior and well-being. In addition to these critical dynamic environmental cues, nutrient digestion rates influence how protein and calories are utilized even though the biochemical mechanisms have not been fully characterized. Since most of the animals' functions, including immunity, are connected to metabolism and energy utilization, it becomes important to determine how to optimize the synchronization of nutrient digestion rates. It is also important to note that the dietary fiber digestion rates we have developed seem to allow us to incorporate dynamics or time into our linear models while maintaining nutrient additivity.

Deciphering the Dietary Fiber Message via Kinetics

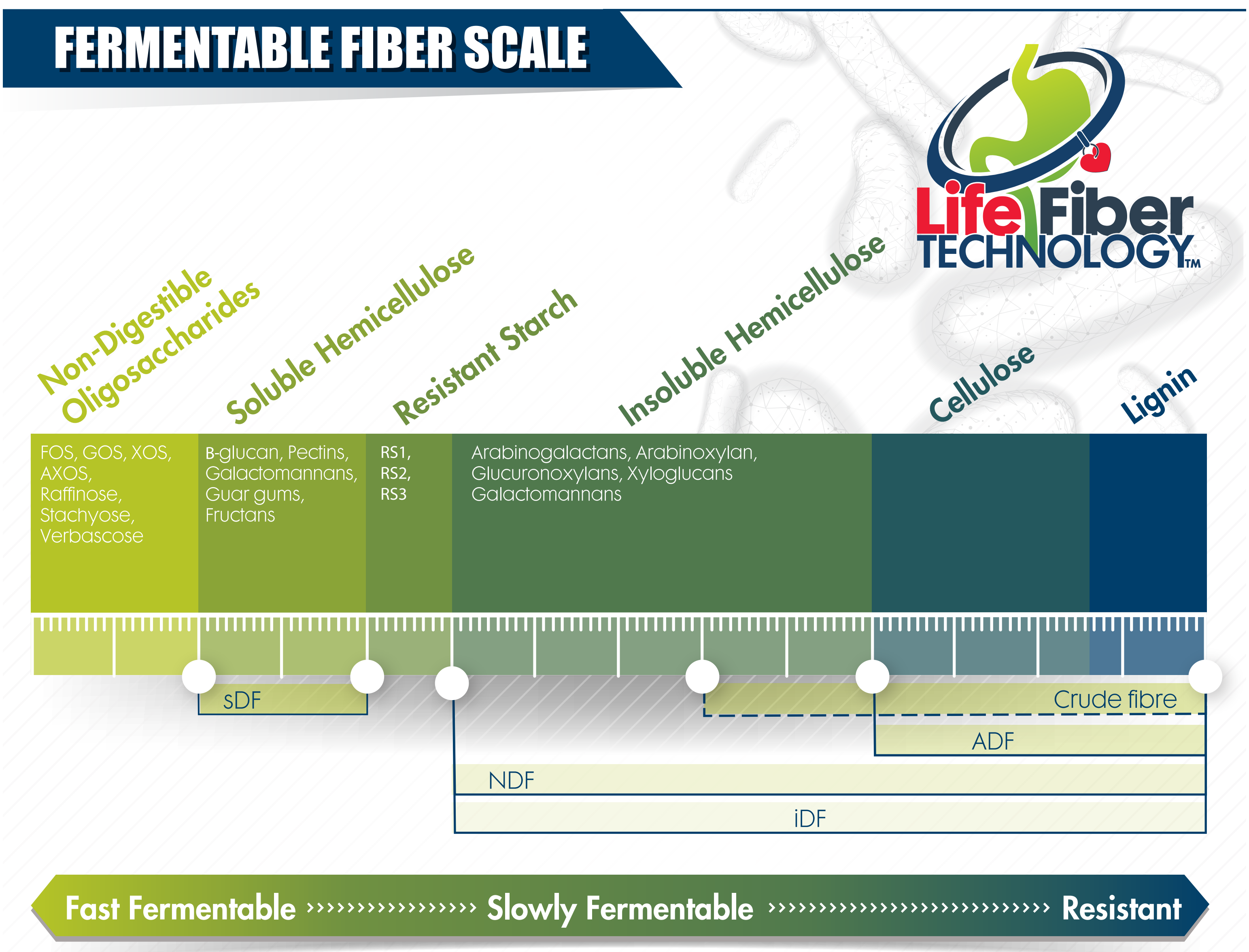
When kinetics of dietary fiber and fermentable load are incorporated into our understanding of analytical data of ingredients, we can begin to unravel not only the response it will elicit in the gut, but also where and when dietary fiber will elicit a response in the gut. Trouw

Nutrition applies this information to diet formulation for optimal animal gastrointestinal health and digesta flow, which is necessary to maintain, and even boost vitality of farm animals and pets. In young animals, for example, we utilize dietary fiber kinetics to achieve three goals.

The first goal is to use rapidly fermentable dietary fiber to help smooth the transition of the young animal from mother's milk, which also contains rapidly fermentable dietary fiber in the form of oligosaccharides and other carbohydrates. This rapid fermentation stimulates bifidogenic microbiota growth, competing with and in the end reducing the proliferation of pathogenic bacteria. The rapid fermentation leads to increased short-chain fatty acid production, most notably butyrate, and reduces the pH of intestinal contents. Butyrate aids in the maturation of the gastrointestinal tract as it serves as the primary energy source for colonocytes.

The second goal is to provide slowly fermentable dietary fiber to the young animal to maintain carbohydrate fermentation throughout the large intestine. Microbes, when given the chance, will ferment dietary fiber over protein because it has a higher energy yield (Diether and Willing, 2019). However, young animals require a protein-dense diet and this can result in undigested protein reaching the large intestine. An unbalanced fermentable load causing excessive proteolytic fermentation can result in large intestine inflammation and lead to diarrhea. In addition, slowly fermentable dietary fiber yields short-chain fatty acids throughout the entire large intestine, thereby reducing colonic pH. Once again, the lower pH throughout the colon limits the growth of pathogenic bacteria, which are mostly responsible for proteolytic fermentation.

The final goal is to optimize intestinal motility, laxation, osmotic balance and stool quality through the utilization of dietary fiber that is resistant to fermentation. Microbes, and therefore, the animal, receive little nourishment from these fiber types, but without it, the intestinal tract would strain to move digesta. Intestinal motility not only aids in laxation, but also stimulates the maturation of the gut through stimulating the regeneration of intestinal



epithelial cells. This type of dietary fiber achieves this by what is referred to as the "scratch factor" by ruminant nutritionists. The resistant dietary fiber scratches (i.e., sloughing) old, inefficient epithelial cells, which are then replaced by new epithelial cells that have a larger surface area, making them more efficient.

Characterizing dietary fiber into rates of fermentation enables the dynamic evolutionary driver [time] to be accounted for in linear feed formulation. This is important because fiber fermentation and microbial growth are nonlinear. This exponential growth of microbial populations oscillate and proceeds continuously through the evolutionary growth phases: lag, exponential, stationary, and death. All of which occur based on time and feed intake patterns and fermentable load. Leveraging this knowledge allows one to counteract negative effects of fermentation occurring in the gut and take full advantage of the positive effects of fermentation.

Dietary Fiber: Code Cracked

Dietary fiber plays a primary role in two key, yet understudied areas in animals: microbial populations and nutrient synchronization. The mystery of the role of dietary fiber in the health of our animals begins to unravel when we add a new dimension to linear diet formulation: time. Dietary fiber fermentation kinetics of ingredients, recently developed by Trouw Nutrition R&D and applied by Milkiwean Nutritionists, allows us to incorporate time into ingredient characterization and, thereby, focus diet formulation for the synchronization of nutrients, enhanced microbial populations, and improved animal performance. Cracking the dietary fiber code thereby, improving animal health and performance.

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