Research, Validation and Commercialization of Technologies

Part 1 of 5: Plausible Scientific Evidence of the Efficacy of SGP+™ in Bovine Herd Performance through Bovine Ration Management

Lignin, Degraded and Depolymerized Lignin, and Select Synergies from Mastic, Ionic Minerals, and Carob

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CONFIDENTIAL

One may entertain what is plausible by first entertaining that it is possible as per renowned author, Robert A. Heinlen, "Everything is theoretically impossible, until it's done."

Preface: SGP+™ is NOT simply Sugarcane Bagasse, BUT a proprietary formulation containing Nutri-Mastic™ (Mastic Gum, Ionic Minerals, Water), Carob, and Sugarcane Bagasse processed through IFUS's proprietary method, whereby Sugarcane Bagasse at 135-145°F shifts in less than 2-minutes, transforming the material into SGP+™ with lowered temperatures of 92-94°F, and continued cooling to 84-86°F for several minutes within 2000-lb bales.

1. Steam coming off the Sugarcane Bagasse before it is formulated, then processed into SGP+™ in Pic. 1:

   Pic 1:

2. It is said that a picture can be worth a thousand words. Below are pictures of structurally intact 2000-lb bales of SGP+™ freshly produced in Pic 2-1 and 2-2:

Pic 2-1

Pic 2-2

3. Pictures of SGP+™ 6-months after production. Unlike untreated bagasse (or hay) compressed into bales, SGP+™ bales begin to collapse with darkened water produced (an indication of lignin depolymerization by microbes naturally occurring in the Sugarcane Bagasse). Pic 3-1 and 3-2:

   Pic 3-1:

"Under anaerobic conditions, major bacterial decomposition occurs, leading to the formation of black or dark grey colored wastewater [30]," Abdoul Wahab Nouhou Moussa, et.al., "Critical State of the Art of Sugarcane Industry Wastewater Treatment Technologies and Perspectives for Sustainability," Membranes (Basel). 2023 Jul 31;13(8):709. doi: 10.3390/membranes13080709

Normally, this black-water from Sugarcane Bagasse results from pile maceration to prevent spontaneous combustion. This effluent would be filled with Iron Sulfides with a characteristic Sulfide smell. Where the dark color is attributed to Iron, there is NO Sulfide smell in the IFUS facility from the black-water as well as NO water added to prevent combustion as the bales are cool.

Pic 3-2:

“A taxonomic classification of the bagasse metagenome reviews the predominance of Proteobacteria, which are also found in high abundance in other aerobic environments. Based on the functional characterization of biomass-degrading enzymes, we have demonstrated that the bagasse microbial community benefits from a large repertoire of lignocellulolytic enzymes, which allows them to digest different components of lignocelluoses into single molecule sugars.” Wuttichai Mhuantong, et.al., “Comparative analysis of sugarcane bagasse metagenome reveals unique and conserved biomass-degrading enzymes among lignocellulolytic microbial communities,” Biotechnol Biofuels. 2015 Feb 8;8:16. doi: 10.1186/s13068-015-0200-8

The IFUS SGP+™ storage areas routinely have scents of a Starbucks Coffee Shop, and/or a bit of sweetened molasses, and in time just simply a fresh earthy smell.

4. SGP+™ after several years (2012). Unlike untreated Sugarcane Bagasse (of hay), SGP+™ disintegrates into dust as shown by Pic 4-1 and 4-2:

   Pic 4-1:

   Pic 4-2

IFUS has since modified its formulation method and the creation of the dust-like product is now occurring in 18-24 months.

5. Pictures of Untreated Sugarcane Bagasse in Pic 5:

Pic 5:

The Sugarcane Bagasse is hot to the touch.

6. Contrasted to 4-month-old SGP+™ broken apart out of a 2000-lb bale in Pic 6:

   Pic 6:

The SGP+™ is cool to the touch.

7. What's happening? Accelerated lignin degradation and depolymerization!

Sugarcane Bagasse in unlike any grass on earth as it produces high levels of starch. When the Sugarcane Bagasse suspends its growth (typically due to cooler temperatures), the starch converts into sucrose. Once the sucrose is extracted from the Sugarcane, the residual sugars are quickly fermented in the remaining pulp (or Bagasse). Over time, microbes in the Sugarcane Bagasse mat use the remaining nutrients and chemicals to degrade the cellulose, hemicellulose, and lignin. However, this can take years and is particularly exothermic.

If Sugarcane Bagasse is compressed into a fiber board or bale, these products remain nondegradable for decades. For nearly a century, Sugarcane Bagasse was converted into brown paper, cardboard, and insulation board.

As a U.S.D.A. ND-40 fiber, most ranchers and dairymen would be ill-advised to use Sugarcane Bagasse as part of their respective Ration Management. This is clearly established by the non-use of Sugarcane Bagasse by ranchers and dairymen in Southeast Louisiana where both cattle and Bagasse are plentiful.

The obstacle to using Sugarcane Bagasse as part of Bovine Ration is the recalcitrant lignin. However, if the lignin is degraded and depolymerized (as IFUS claims), then Sugarcane Bagasse becomes as per Dr. Pat Bagley, "the holy grail of energy and nutrition for bovines." Dr. Bagley is the former Dean of Tennessee Tech School of Agriculture and now serves as Director, SUAREC Beef Research Unit as well as Acting Vice Chancelor of Institutional Development. He is one of the authors of LA HCR-42 2024, commonly referred to as the Bagasse Utilization Bill.

IFUS holds that is has discovered a commercially viable process by which lignin from Sugarcane Bagasse can be degraded and depolymerized *in vitro* prior to bovine consumption making Sugarcane Bagasse, when combined with Mastic Gum, Carob, Ionic Minerals, and Water, a potential power food…one IFUS named SGP+™.

Furthermore, Manure Scoring coupled with Herd Performance as reported to IFUS by ranchers and dairymen blending SGP+™ with the respective rations, strongly suggests further degradation and depolymerization of the lignin *in vivo.*

"Manure scoring determines supplementation needs,"

> "When combined with other estimates such as forage availability and quality, a diet can be quickly changed to meet the cow's nutrient

requirements rather than waiting for body condition to fall low enough that the producer will notice a change. Manure scoring can indicate the quality of nutrition a cow has had in the past one to three days, while body condition score will indicate the nutritional history of the past several weeks to months.

“Manure is scored on a 1 to 5 basis, with a score of 1 being very fluid and 5 being extremely dry and segmented. The next few paragraphs will detail each score and associated diet quality. Reference photographs have been included with approximate levels of dietary protein and energy (TDN) listed.

“A manure score of 1 is of cream soup consistency. It can indicate a sick animal or a highly digestible ration that contains excess protein, carbohydrates or minerals, and low fiber. The addition of hay will slow down the rate of passage and thicken the manure.

“Score 2: >20% CP; >68% TDN of diet

“Manure that will score a 2 doesn’t stack; the pat is usually less than 1 inch thick and will lack consistent form. This manure has the consistency of cake batter. Excess protein, carbohydrates and low fiber characterize the diets that produce this manure. Rate of passage is very high, and adding hay to this diet will slow it down to allow for more absorption in the intestinal tract.

“Score 3: 12-15% CP; 62-70% TDN of diet

“Manure score 3 is ideal and will typically start to take on a normal pat form. The consistency will be similar to thick pancake batter. It will exhibit a slight divot in the middle. The pat will be deeper than a score 2 pat, but will not stack. This diet is not lacking nutritionally, yet is not in excess for the cow and her physiological stage.

“Score 4 manure is thick and starting to become somewhat deeper, yet is not stacking. The consistency of the manure will be equivalent to peanut butter. This manure indicates a lack of degradable rumen protein, excess low quality fiber or not enough carbohydrates in the diet. Supplementation of additional protein with high rumen-degradable protein can increase total diet digestibility. Cottonseed meal and soybean meal are excellent sources of this type of protein.

Score 5: <6% CP; <55% TDN of diet

“The highest and least desirable score is 5. This manure is firm and stacks over 2 inches in height. It will also have clearly defined segments and is very dry. This manure indicates the cow is eating a poor quality forage diet that is inadequate for protein and carbohydrates, and high in low quality fiber. Rate of passage has slowed down to the point that excess water has been reabsorbed in the intestines. The rancher will need to consider additional supplementation to meet the cow’s protein and energy requirements.”

Dr. Robert Wells, formerly of the Noble Research institute is now “Professor of Practice and the Paul C. Genho Endowed Chair in Ranch Management, Beef Cattle Management and Ruminant Nutrition. Dr. Well, PAS is a South Texas native who has spent his career consulting with ranchers to meet their ranch management and profitability goals across the Southern Great Plains and internationally. Wells earned a bachelor’s degree in Animal Science from Stephen F. Austin State University, his Master of Science degree with a focus on ruminant nutrition from Texas Tech University, and his Ph.D. from the University of Illinois in beef cattle management and ruminant nutrition.”

For purposes of comparison, here is a side-by-side demonstration of manure pats provided by the Noble Research Institute. Noble's Mission is to "To guide farmers and ranchers in applying regenerative principles that yield healthier soil, more productive grazing land, and business success."

Score 3 Manure Pat: Deer Run Ranch with 90%SGP+™ / 10% Cracked Corn:

Manure Pats from Joe Wilcox as he pushes SGP™ to 92% with 8% DG. Notice pats moving from Score 3 to Score 3.4/4.0. Hence, Joe will back down to 90% SGP+™/10% DG until Score 3 pats return. Joe is conducting his 4th trial (a blind trial this round), whereby he bulked half the herd on SGP+™ to sell weight in 60 days vs. the control group at 90+ days.

Manure from India with SGP+2.0™ at only 15% of ration mixed with Sugarcane Tops and Protein Concentrate. Also, during the 15-day trial, Manure Score Improved. Furthermore, where Milk fat percentage was the same, SNF (Solid-non-fat) percent increased by 0.6-0.8%. SNF typically ranges around 8%. Hence, for this Indian Trial, the 0.6-0.8% increase moved their respective milk fat to 7.5%. This provide to be an approximate increase of 10.0% increase in total SNF in their milk. The substantive nature of this cannot be understated considering that milk and milk-based products are the primary source of nutrition in India

Hence, IFUS offers these thoughts and this evidence for your consideration.

Day 1 Manure

Day 15 Manure

To repeat: "When combined with other estimates such as forage availability and quality, a diet can be quickly changed to meet the cow's nutrient requirements rather than waiting for body condition to fall low enough that the producer will notice a change. Manure scoring can indicate the quality of nutrition a cow has had in the past one to three days, while body condition score will indicate the nutritional history of the past several weeks to months."

1. I.V.T.D. (*In Vitro* Total Digestibility) results of Sugarcane Bagasse as compared to SGP+™:
    a. Testing performed by the L.S.U. Southeast Research Station Forage Quality Lab demonstrated a marked I.V.T.D. improvement of SGP+™ as compared to Sugarcane Bagasse (47.5% vs 32.0%, respectively). Where by this analysis alone SGP+ should NOT produce the Herd Performance claimed by ranchers and dairymen, per Dr. Mike McCormick, it does beg the question as to what caused this significant improvement in I.V.T.D.
2. Lignin Analysis on Sugarcane Bagasse as Compared to Manure Lignin Analysis on Score 3 Manure Pats from a beef herd fed 90%SGP+™/10% Cracked Corn:
    a. Forage / Manure Analysis Comparison suggests SGP+™ is being digested and producing both CP and TDN required for Bovine Herd Performance.
    b. A series of Forage analyses performed by L.S.U. Southeast Research Station Forage Quality Lab showed Sugarcane Bagasse used in the formulation of SGP+™ to contain roughly 28% lignin.
    c. However, the lignin concentration decreased to 19% in an 85% SGP+™/15% Cracked Corn ration mix (as analyzed by Cumberland Valley Analytical Services).
    d. Furthermore, in Manure Analysis performed on Score 3 Manure Pats resulting from the 85/15 mix, showed a further decrease in lignin content to 7-9% (as analyzed by Cumberland Valley Analytical Services).
    e. Cumberland also noted undigested cracked corn in the original manure sample. Of interest Sugarcane Bagasse (SB) is high in S-Lignin, where Corn is high in G-Lignin. Studies indicate that White-Rot Fungi and other microbes naturally found in SB target the S-Lignin for degradation and depolymerization.
3. Manure Analysis performed by Texas A&M on 80%SGP+/20%DG (Distillers Grain):
    a. "Cow Weight and Body Condition: All sampled cows weighed around 1,250-1,300 lbs and had a Body Condition Score ~6 (on a 1-9 scale). A BCS of 6 means they were in good flesh - neither too thin nor overly

fat, which is ideal for cows nursing calves. Maintaining this condition indicates the feed is keeping them healthy."

   i. Of note, is that these Score 6 cows were purchases as Score 1 & 2.

b. However, other parts of the A&M analysis were reached based on a misunderstanding that SGP+™ is NOT untreated Sugarcane Bagasse.
c. The A&M report continually refers to the SGP+™ as "bagasse". SGP+™ uses bagasse as one of multiple ingredients. And, based on photographic and microscopic analysis, untreated Sugarcane Bagasse is VERY different from SGP+™. This confusion resulted from a miscommunication as to the nature of SGP+™.
d. Furthermore, the A&M analysis concludes that the results indicated that more study of SGP+™ is warranted due to seemingly unexpected positive Herd Performance outcomes.

Hence, if one considers (1) the photographic evidence presented above of the transformation of untreated Sugarcane Bagasse(SB) as compared to SGP+™, (2) I.V.T.D. improvements from untreated SB as compared to SGP+™, (3) Forage/Manure Analysis demonstrating a trend line of decreased lignin concentration when compared to untreated SB to SGP+™ to manure, (4) improvements to Manure Score 3 pats, and (5) reported improvements in overall Herd Performance, then IFUS holds that where present analysis methods in isolation cannot explain the efficacy of SGP+™, when taken as a series of data points and interconnected from starting ingredients to Herd Performance the overall trajectory demonstrates that:

1. Lignin in the Sugarcane Bagasse (SB) when transformed into SGP+™ is being degraded and depolymerized.
2. This SGP+™ is in fact being converted into CP and TDN as indicated by Score 3 Manure Pats and Herd Performance.
3. SGP+™ has demonstrated efficacy in the improvement of Herd Performance.

In consideration of this information, IFUS can provide deeper and plausible scientific explanations in support of the efficacy of SGP+™ as compiled in the competed White Paper below:

IFUS Table of Contents: Plausible Scientific Evidence of SG+™ in Bovine Herd Performance through Ration Management Part 1:

IFUS Point 1: **QUALIFYING IFUS's THINKING:** Before any comment is offered on the Manure Analysis and Report performed and written by professionals at Texas A&M, the IFUS Scientific Team wishes to clearly establish its respect for those who study, work, teach, and/or research at Texas A&M now or in the past. The team holds that its experience with the Aggie Community has generated this respect. Furthermore, the IFUS Scientific Team is deeply encouraged by reports from the Schmidt's that indicate A&M's willingness to further investigate the efficacy of SGP+™.

IFUS Point1a: To that end, the IFUS Scientific Team would we willing and eager to share what it thinks it knows about SGP+™ as reflected in the comments below. To this point, the IFUS Scientific Team receives reports from ranchers and dairymen applying SGP+™ as part of their ration management. These ranchers and dairymen send pictures and/or videos (and sometimes data) making claims regarding their respective herd's performance. Our team hears this over and over, then begins to investigate, wondering if the claims are real and if so why. The information shared below is offered in that vein as not a single member of the IFUS team EVER imagined any of these reports to be possible. Yet, the science is telling us that these claims are not only possible, but plausible (to our amazement). Hence, our quasi-forensic-like search for the truth continues.

> IFUS Point 1a(1): As a point of consideration SGP+™ was designed to use Sugarcane Bagasse as a delivery system for Nutri-Mastic™/Carob as a supplement to Bovine Ration Management. Initially 15% SGP+™ to 85% Ration was advised. When ranchers began moving the percent of SGP+™ to Ration upward, the IFUS Scientific Team vehemently objected and discouraged ranchers from doing so. None of our team would feed their beef or dairy cows Sugarcane Bagasse. Hence, the thoughts offered below were NOT considered possible at the time. Today, ranchers and dairymen are finding success in Herd Performance with Ration Mixes as high as 92%SGP+™/8% Other Ingredients, to the IFUS's Scientific Team's amazement. Hence, the quest to understand how and why.

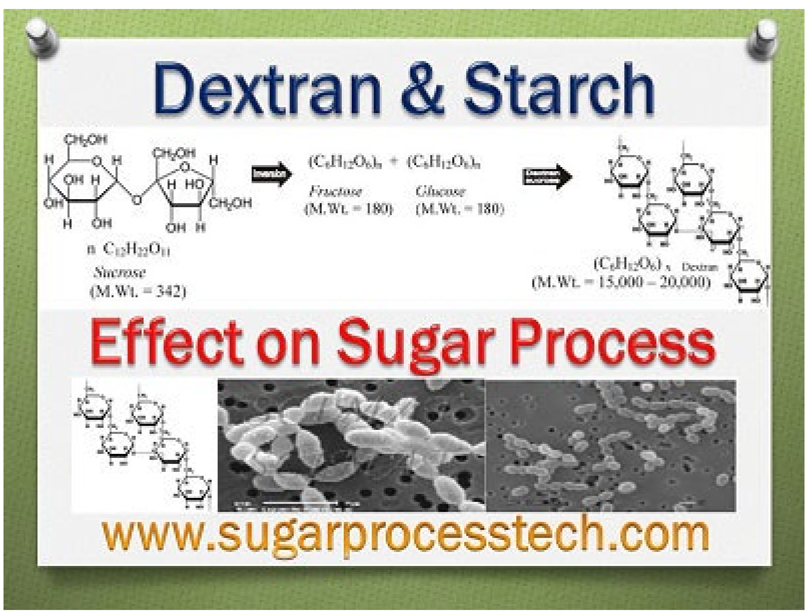
IFUS Point 1b: Also, for purposes of this discussion, IFUS will focus primarily on the lignin in Sugarcane Bagasse. However, SGP+™ uses Sugarcane Bagasse as one of several ingredients. These ingredients include Nutri-Mastic™ (a proprietary formulation of Mastic, Ionic Minerals, and Water) plus Carob…all of which play essential rolls through scientifically demonstrated synergies when overlayed with Chemistry (Organic/Inorganic/Physical), Biochemistry, Polymer

Chemistry, Thermodynamics/Kinetics, Virology, Bacteriology, Mycology, and even a dash of Geology/Geophysics.

IFUS Point 1b(1): Furthermore, the residual sugars to include ISP, Dextran and others, along with the ash content, the vitamins and minerals (to include high levels of iron and manganese), the waxes, gums, and fatty acids, and other chemical components contained within Sugarcane Bagasse are for the most part ignored as the discussion below is complex enough without offering this added science. However, at times overlaps/synergies/symbioses become most apparent when various components are overlayed one over the other…much like that which any biochemical cycle reveals in its individual action and interaction with other cycles. (As a funny, this drives the IFUS Scientific Team wild, cross-eyed, and simply bonkers!)

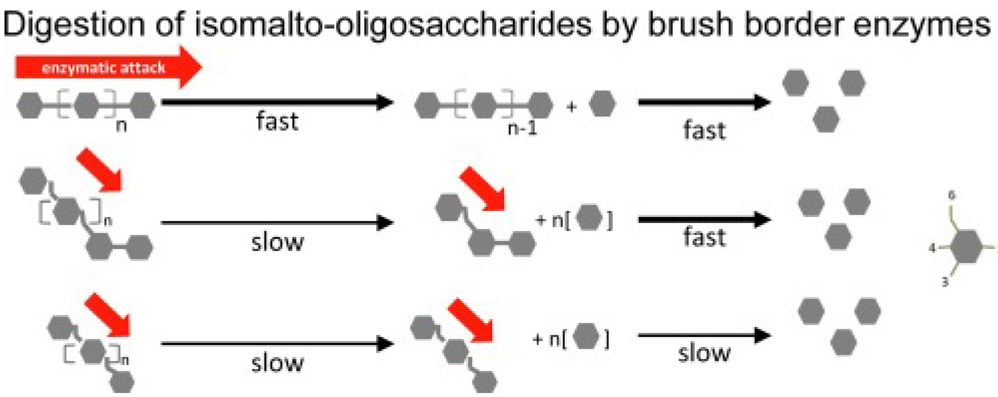
IFUS Point1b(2): As an example of one aspect of the added science, Dextran is produced in Sugarcane processing by a gram-positive bacteria, *Leuconostoc mesenteroides*. "As a facultatively anaerobic bacteria, it thrives in both the presence and absence of oxygen."

Dextran notoriously produces elongate polymeric grains of glucose units as shown in the diagram below.



"Dextran, a renewable hydrophilic polysaccharide, is nontoxic, highly stable but intrinsically biodegradable. The α-1, 6 glycosidic bonds in dextran are attacked by dextranase (E.C. 3.2.1.11)." Furthermore, "Dextranases (α-1,6-D-glucan-6-glucanohydrolase; EC 3.2.1.11) randomly hydrolyze α-1,6-linkages of dextran and release isomaltooligosaccharides (IGs) of various sizes [4]. It is extremely rare that the product of hydrolysis is glucose. which is an inducible enzyme."

However, of interest is that the IGs are shown to have high beneficial effects on mammalian digestion and absorption and are thought to be metabolized as shown by, "Current in vitro digestibility assays for dietary fiber including resistant starch and non-digestible oligosaccharides use pancreatic amylase and fungal amyloglucosidase (Table 1), which hydrolyze linear oligosaccharides with α-(1 → 4) and α-(1 → 6) linkages (McCleary, 2019, Pazur and Ando, 1960)."



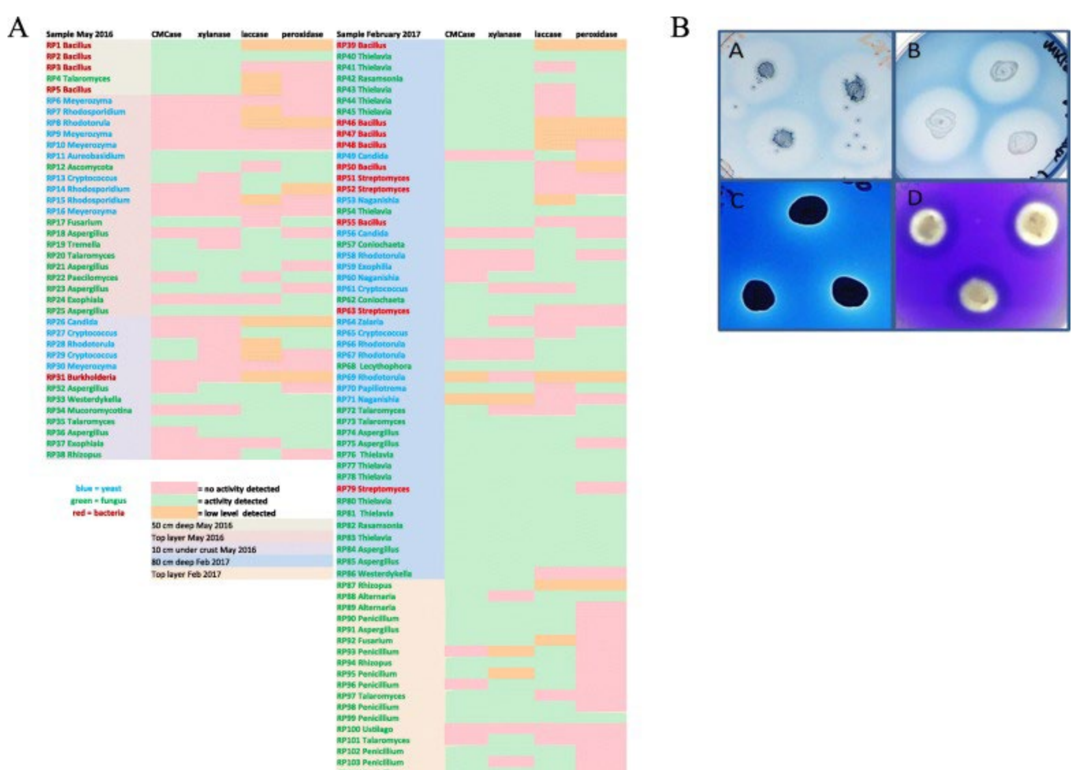
Furthermore, any number of studies out of Brazil clearly establish a chemical/biochemical pathway through which Dextran is converted into IG's, reinforcing Sugarcane Bagasse as "the Holy Grail of Energy and Nutrition for Bovines", notwithstanding the recalcitrant lignin. These studies focus on Dextranases produced from microbes (plant, fungal, and bacterial) similar to those found in marine environments.

Furthermore, Dextran and other components of Sugarcane Bagasse contain and/or can produce Xylan, which in turn can be naturally transformed by microbes in the SB. "Xylan is one of the foremost anti-nutritional factors in common use feedstuff raw materials. Xylooligosaccharides produced from xylan are considered as "functional food" or dietary fibers[13] due their potential prebiotic properties.[14] Broekaert, W.F., et.al, (2011). "Prebiotic and Other Health-Related Effects of Cereal-Derived Arabinoxylans, Arabinoxylan-Oligosaccharides, and Xylooligosaccharides". Critical Reviews in Food Science and Nutrition. 51 (2): 178–194. doi:10.1080/10408390903044768. PMID 21328111. S2CID 205689400.

As a case in point, "Sugarcane processing roughly generates 54 million tonnes sugarcane bagasse (SCB)/year, making SCB an important material for upgrading to value-added molecules. In this study, an integrated scheme was developed for separating xylan, lignin and cellulose, followed by production of xylo-oligosaccharides (XOS) from SCB." "Extraction of sugarcane bagasse arabinoxylan, integrated with enzymatic production of xylo-oligosaccharides and separation of cellulose," Leila Khaleghipour, et.al., Biotechnol Biofuels. 2021 Jul 3;14:153. doi: 10.1186/s13068-021-01993-z

Furthermore, the complexity of the microbial genome in Sugarcane Bagasse is complex at best as evidenced by "The variety of species that was found and that are known for biomass degradation shows that the bagasse pile was a valuable selective environment for the identification of new microbes and enzymes with biotechnological potential. In particular, lignin-modifying activities have not been reported previously for many of the species that were identified, suggesting future studies are warranted." "A snapshot of microbial diversity and function in an undisturbed sugarcane bagasse pile," Leigh Gebbie, et.al, BMC Biotechnol. 2020 Feb 28;20:12. doi: 10.1186/s12896-020-00609-y

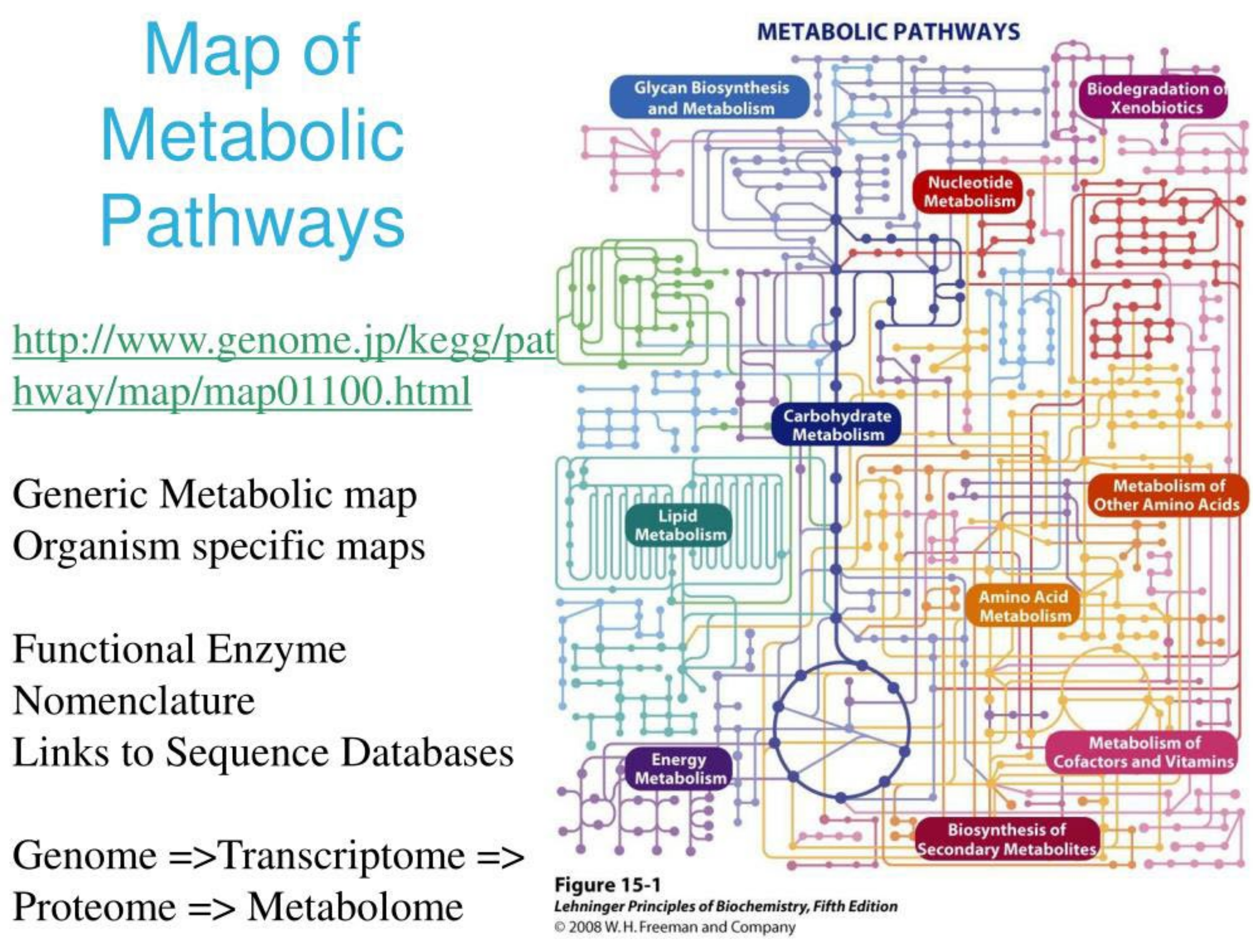
Fig. 2.



Screening of bagasse microbes for biomass-degrading enzymes. **a** The bacteria, yeast and filamentous fungi (RP1-RP103) isolated from different positions in the pile were screened for cellulase, xylanase, laccase and peroxidase on carboxy-methyl cellulose with trypan blue dye, azo-xylan, remazol brilliant blue and azure B containing agar plates, respectively. Activity was scored as positive or not based on clearance zones around the colonies. Low activity indicates a very minor clearance zone that took longer than 7 days to become visible. Examples of each assay are shown in **b**

> The key to unlocking all of this science and more is the degradation and depolymerization of lignin. And, present Forage and Manure Analysis in isolation are incapable of demonstrating this degradation and depolymerization as well as any plausible shift in Bovine Ration Management proposed due to the degradation and depolymerization both *in vitro* and *in vivo.* However, if managed against a "Data Grouping" (especially one from a forensic point of view), Forage and Manure Analysis (as well as other established methods) may be found to be invaluable tools in understanding and creating a C-Change in Ration Management thinking, especially where SGP+™ is involved.

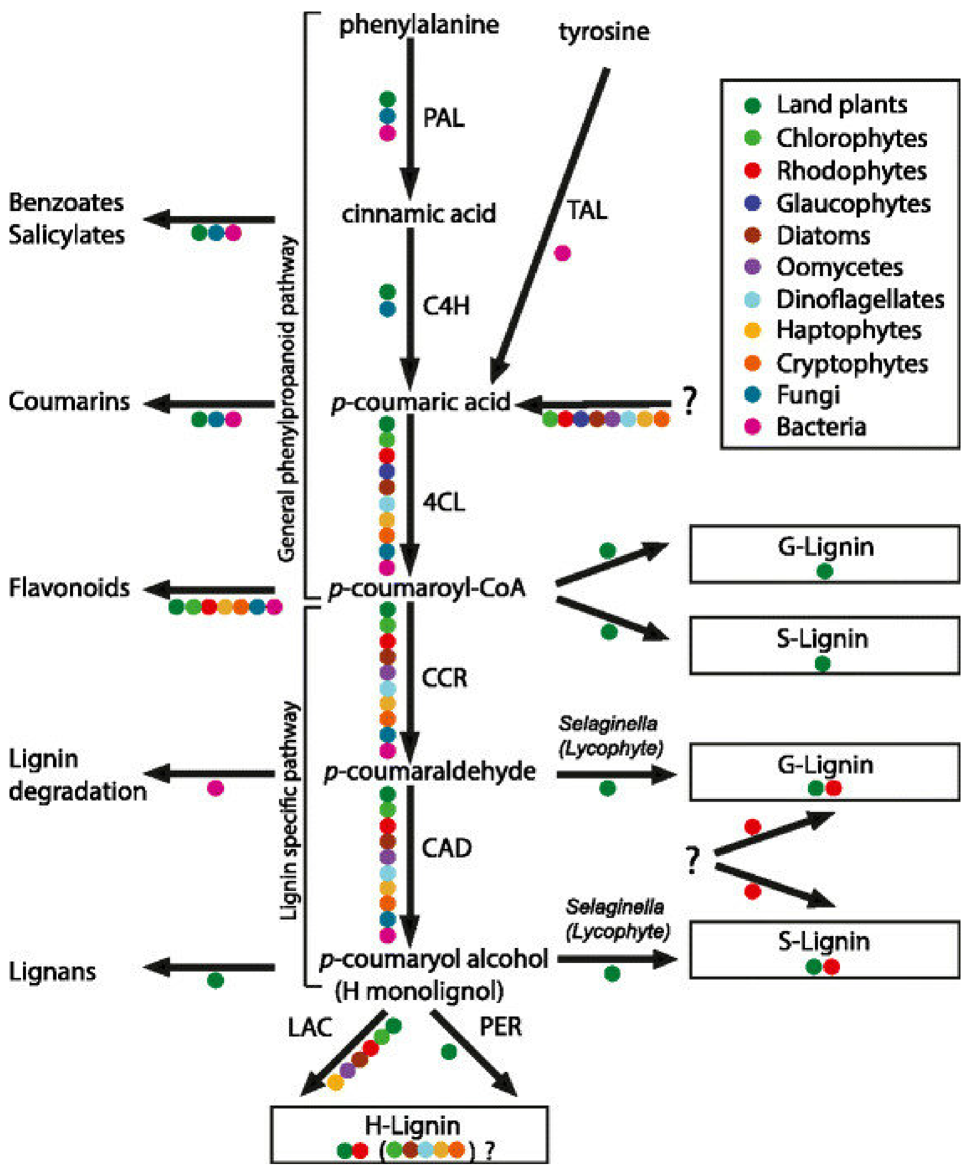
IFUS Point 1c: The diagram below reflects the complexity of that which the IFUS Scientific Team is attempting to understand as it relates to Mammalian metabolism. When compared to Bovine metabolism, the complexity of where, when, and how increases exponentially as you know. (Hence, the bonkers part.)



IFUS Point1d: Additionally, when one considers the suggested lignin degradation process , then one finds added layers of complexity, even when ignoring the Mastic, Carob, ionic minerals, and Water (individually and collectively added to the formulation that transforms Sugarcane Bagasse into SGP+™), as well as the rheology of the actual formulation processing, which affects the thermodynamics and kinetics of transformation into SGP+™.

Also, note the production of "flavonoids" is the diagram below. "Flavonoids" are used in perfume production. Many flying insects (particularly biting flies and gnats) are repelled by these compounds…creating yet another possible synergy.

Furthermore, the diagram below illustrates yet another synergy in the production of the Salicylates (pain and inflammation reducers) and Benzoates (topical antiseptics / decongestants / selected anti-fungals / etc.).



IFUS Point 1e: Hence, one could propose that all things written below are theoretical and possibly even speculative. However, when placed into a "Scatter Plot" with interconnecting lines and overlays, an image forms that seems more than speculation or coincidence, especially when reconciled to Manure Scoring and reports by ranchers and dairymen as to Herd Performance. Here is a list of the kind of claims being made to IFUS from ranchers and dairymen applying SGP+™ as part of their respective Ration Management:

- Improved overall health and wellness of their heifers as indicated by Herd Scoring.
- Improved health and wellness of progeny born within their herds as reflected in decrease infant mortality.
- Improved estrous cycle amongst their heifers as indicated by the ease of fertilization and number of calves born / year.
- Reduction in the quantity of high-priced feed stocks fed to their heifers (e.g., grains, hay, supplements) as indicated by their cost, revenue, and profit margins.
- Reduction in high-energy "junk-food" (e.g., chicken waste, candy fall-off, etc.) fed to their heifers as indicated by their application of 90+% SGP+™ to either DG or Cracked Corn plus foraging.
- Reduction in antibiotic application and other medications again as indicated by their financial costs.
- Reduction of flies on the cattle and fly larvae in the manure.
- Improvement in heat tolerance and hydration of their heifers as indicated by their reduced water consumption, observable decrease in urinary output, and survival rates of both adults and calves.
- Improvement in muscle mass, milk production and quality (specifically an improvement in NDF), finish, and overall quality of their heifers as indicated by pictures of milk bags, colostrum dripping from teats, health of the calves, DWG, increased sale price with improved Scoring, etc.
- Improved quality and taste of meat as indicated by comments from butchers grading the meat, the marbleization, and the fact the meat just tasted "damn good!".
- A negative impact on weight gain and sperm-count of bulls supporting claims of natural estrogenic properties.

IFUS Pont 1f: For purposes of expediency, the IFUS Team is now focused on (1) continually updating the thinking reflected in this "White Paper" as new data is presented to IFUS combined with its continued review of scientific studies, and (2) offering thinking so as to address the Screw-Worm Fly issue confronting the U.S. (with this work already underway, and now a primary focus.

IFUS Point 1g: Lastly, in reading this "White Paper" (for lack of better words), if a typo is found, we beg your forbearance as this particular White Paper (although based on a decade-plus of work) has been put together in a very short time frame to specifically respond to the good work performed and interest indicated by the A&M Team, and at the request of Deer Run Ranch.

IFUS Point 2: **A PERSPECTIVE ON MEASUREMENT & SCIENCE:** The folks who perform Forage, Manure, and other such analyses are required to use established methods generally accepted globally and supported by decades of proof. With that said, it seems prudent to pause and consider for what purpose these analytical methods were designed. History suggests that to assist in an emerging need to feed the masses as people left the family farm and congregated into cities, science had to evolve. This migration from the family farm to the city was accelerated by the Industrial Revolution in combination with the U.S. Civil War, WWI, then WWII. Hence, the analytical methods used to date support Ration Management, designed as such with little consideration about Greenhouse Gases, the exhaustion of top soil, the application of synthetic fertilizers/pesticides/herbicides/etc, and now what is being reported as an unhealthy cow.

IFUS Point 2a: Additionally, is would also seem prudent to again reflect on the nature of measurement. Herd Performance is the end all tell all as it is the ultimate Performance Measurement. Forage Analysis (Input), Manure Analysis (Output), and the like are derived from some direct measurement as well as interpolation, extrapolation, and applied normalization constants. The data that results would be a blend of Process Indicator and Process Measurement…both of these begging the question, "As compared to what specifically?" Furthermore, measures of this kind were designed with the notion of "understand your forage, then supplement from there". SGP+™ is NOT a mere forage-to-forage replacement (as we will discuss later).

> **IFUS Point 2a(1):** As an example of the limitation of measurement**,** **"**Table 1. The ruminal pH scale cannot determine the concentrations of individual components. The pH scale reflects the "quotient" between acids and bases, not absolute concentrations. The Henderson-Hasselbalch equation

demonstrates this concept. Two solutions can have similar pH values despite different concentrations of specific components."

Henderson-Hassellbalch equation.

$$pH = pKa' + Log\,[HCO_3^-/\,dCO_2]$$

	Solution A		Solution B	
Molecules	HCO_3^-	dCO_2	HCO_3^-	dCO_2
Concentrations (mM)	100	100	10	10
pH	pKa'		pKa'	

Ruminal pKa' = 6.1 (Hille et al., 2016)

IFUS Point 2b: However, since these measurement systems were created, there have been emerging repercussions. As an example: "Man became distracted from the importance of organic compound cycling when it was discovered that soluble acidic based N P K "fertilizers" could stimulate plant growth. Large industrial concerns took advantage of the N P K discovery to market industrially processed "fertilizers" from mineral deposit. Continued use of these acidic fertilizers in the absence of adequate humic substances (in the soil) has caused many serious sociological and ecological problems. Man needs to reconsider his approach to fertilization techniques by giving higher priority to soil humus.", ORGANIC MATTER, HUMUS, HUMATE, HUMIC ACID, FULVIC ACID AND HUMIN: THEIR IMPORTANCE IN SOIL FERTILITY AND PLANT HEALTH, Dr. Robert E. Pettit, Emeritus Associate Professor Texas A&M University

IFUS Point 2b(1): As a note, as these mineral deposits were depleted (as well as light/sweet crude oil), chemicals processed from complex "heavy/sour crude oil" filled the mineral void.

IFUS Point 2c: To Dr. Pettit's point, a study was performed on Humic Substances (HS): "Effects of a dietary complex of humic and fulvic acids (FeedMAX 15™) on the health and production of feedlot cattle destined for the Australian domestic market. Conclusions: Feeding the humic and fulvic acid complex, FeedMAX 15™, at 0.055 g per kg body weight per day, can increase growth rate and feed conversion efficiency in feedlot cattle." P M V Cusack, Aust Vet J., 2008 Jan-Feb;86(1-2):46-9.

IFUS Point 2c(1): Furthermore, another study concluded that, "…the addition of HS to the diet of beef heifers resulted in a favorable increase in the retention of N, with increased NH3-N and protozoa counts at low to moderate doses of HS. HS favorably increased the digestibility of CP and the retention of N, and decreased fecal N excretion. The addition of HS to the diet had no effect on CH4 production and the microbiome was altered in a manner that was consistent with the lack of change in CH4 production. Further study should assess the effects of HS additive on growth performance in feedlot cattle." Effect of humic substances on rumen fermentation, nutrient digestibility, methane emissions, and rumen microbiota in beef heifers, Stephanie A Terry, et.al., J Anim Sci. 2018 Aug 29;96(9):3863–3877. doi: 10.1093/jas/sky265.

IFUS Point 2d: As one might expect, Sugarcane bagasse, especially degraded Sugarcane bagasse, is shown to produce both humic and fulvic acids. "Submerged fermentation as a suitable solution to produce humic and fulvic acids from sugarcane bagasse," H. Ghanavati, et.al., doi 10.24200/sci.2022.57665.5357

IFUS Point 2d(1): IFUS pretreats Sugarcane bagasse with Nutri-Mastic™ and Carob, then processes the formulation in a proprietary method to degrade, and initiate depolymerization of the lignin (See IFUS Point 4c).

IFUS Point 2e: Furthermore, there is significant scientific evidence that synthetic fertilizers, pesticides, and herbicides are negatively affecting humic and fulvic acid production. Where such fertilizers, pesticides, and herbicides are used on Sugarcane, the processing of the Sugarcane into bagasse and subsequent storage outdoors with continued required hydration allows for many of these chemicals to be washed, degraded, and/or vaporized away.

IFUS Point 2c(1): Hence, the remaining sugars, waxes, iron and other ingredients in Sugarcane bagasse begin to immediately ferment, by which maceration of the cellulose, hemicellulose, and lignin is initiated. Subsequently, the formulation into SGP+™ further accelerates this degradation and depolymerization of the lignin. (See IFUS Point 4)

IFUS Point 2c(2): This accelerated fermentation produces HS to include Humic and Fulvic acid, all of which is linked to lignin degradation and depolymerization. "Sugarcane bagasse (SCB) which is a waste byproduct of sugarcane industry can be used as soil amendments to improve crop yield and provide reasonable economic means to recycle these wastes in an environment friendly manner. In this study experiments were conducted to test the rate of production of humic acid during the decomposition of SCB for 30 days and effect of SCB amendments on growth components of Chinese Cabbage (Brassica rapa, subsp. pekinensis)." Mwita Solomon Chacha, et.al., Soil Amendments with Sugarcane Bagasse and its Effect on Soil Humic Acid Contents and Chinese Cabbage Growth Components, Agri Res & Tech: Open Access J 21(3): ARTOAJ.MS.ID.556166 (2019).

IFUS Point 2f: Many of the synthetic chemical compounds applied as fertilizers (to include NH4), herbicides, and pesticides, also destroy natural microbes, once part of the bovine's ration and the source of natural protein. "How Does a 1,200 Pound Cow Get Enough Protein?" Sam Westreich, PhD, Sharing Science, Aug 27, 2018. Additionally, these synthetic chemical compounds often disrupt or destroy many of the natural probiotic compounds required for natural and optimal herd health.

IFUS Point 2f(1): This is NOT to suggest that some of the synthetic compounds have only caused problems with no benefits. However, excessive and constant use of these, to push the land and the cow, has created new problems that are proving significant at the least. Drug resistant microbes and pesticide resistant bugs/insects are just two examples…not to mention the accumulation of these synthetic chemicals in the fat and tissue structure of the bovine itself.

IFUS Point 3a: **PROTEIN:** The initial question these days is, "What is your Crude Protein?" As per the recent adage, "Answer only important if know right question." Hence, considering the drivers impacting today's ranching and dairy operations, this is absolutely the "WRONG" question. A deeper understanding of the role of lignin, and more so degraded/depolymerized lignin is required to illuminate this point.

IFUS Point 3a(1): Where bodybuilders and athletes once bombarded their systems with protein, many have reduced their protein intake by 80% with some becoming vegetarians. These athletes report reduced acidosis, improved kidney and liver function, and a myriad of other health improvements, while improving lean muscle mass as well as improved cycle-time to recovery.

IFUS Point 3b: The very present-day Ration Management Strategy and supporting technologies have seemingly run the course in that herd health and performance, Greenhouse Gas Production, Top-Soil/Mineral Depletion, and a myriad of economic drivers require a C-Change in Ration Management thinking, analysis, and formulation. The cow and the Earth are both screaming at us and telling us this is so…as is a rash of new diseases and health conditions impacting human life. Throwing more "stuff" at the cow is NOT the answer!

IFUS Point 4a: **SGP+™ & LIGNIN:** Hence, quite by accident (or maybe providence), Deer Run Ranch (as well as several other successful ranching operations) are applying SGP+™…a product produced using Nutri-Mastic™ (Mastic Oil Emulsified in Ionic Minerals and Water), Carob, and Sugarcane Bagasse.

IFUS Point 4b: SGP+™ is NOT simply Sugarcane Bagasse. Here's why:

IFUS Point 4c: Formulating SGP+™ demonstrates immediate shifts in steaming Sugarcane Bagasse (135-145°F) in less than 2-minutes, transforming the material (SGP+™) to lowered temperatures (92-94°F), with continued cooling (84-86°F) for several minutes within 2000-lb bales. Lignin depolymerization is shown to be endothermic. Furthermore, the sheer introduced in the process is shown to degrade the lignin. Lignin degradation and depolymerization are very different physical and chemical processes. However, the synergies between the two are critical in understanding SGP+™.

IFUS Point 4d: Testing performed by the L.S.U. Southeast Research Station Forage Quality Lab demonstrated a marked I.V.T.D. improvement of SGP+™ as compared to Sugarcane bagasse (47.5% vs 32.0%, respectively). Where by this analysis alone SGP+ should NOT produce the Herd Performance claimed by ranchers and dairymen, per Dr. Mike McCormick, is does beg the question as to what caused this significant improvement in I.V.T.D.

IFUS Point 4e: Like hay, when Sugarcane Bagasse bales are stacked one atop the other, the structural integrity of the bale remains intact for ages. This is due to the lignin. However, when SGP+™ bales are stacked one atop the other, within 90-days the bales collapse. Change to the fiber structure and feel of the fiber becomes apparent. Examination under microscopy also illustrates a shift in fiber morphology.

IFUS Point 4f: Numerous scientific studies establish the polymerization of lignin from only three monomers. "Heterogeneity arises from the diversity and degree of crosslinking between these lignols. The lignols that crosslink are of three main types, all derived from phenylpropane: (1) coniferyl alcohol (3-methoxy-4-hydroxyphenylpropane; its radical, G, is sometimes called guaiacyl), (2) sinapyl alcohol (3,5-dimethoxy-4-hydroxyphenylpropane; its radical, S, is sometimes called syringyl), and (3) paracoumaryl alcohol (4-hydroxyphenylpropane; its radical, H, is sometimes called 4-hydroxyphenyl)."

IFUS Point 4f(1): Lignin in Sugarcane contains all three lignols, which gives both the lignin and subsequently the stalk a unique morphology.

IFUS Point 4g: Lignin accounts for 22-30% of bagasse's composition, yet is shown to possess high antioxidant and antimicrobial (non-probiotic) activity. Three morphological forms of lignin exist: H, G, and S. Bagasse lignin is S-rich (H:G:S 2:38:60, respectively). (Dr. Fred Martin, retired and formerly at L.S.U.) may be the world's single expert on breeding Sugarcane for specific locations and soil types…all of which impact the H/G/S ratios. Just as an FYI, the H/G/S ratio of the sugarcane affects the "Mill Settings" of the raw sugar mill as well as the required maceration requirements to extract sugar from the fiber, etc.

IFUS Point 4g(1): As an aside, the antimicrobial properties of SGP+™ were substantiated by testing conducted by Russell Marine Group on *e-Coli*.

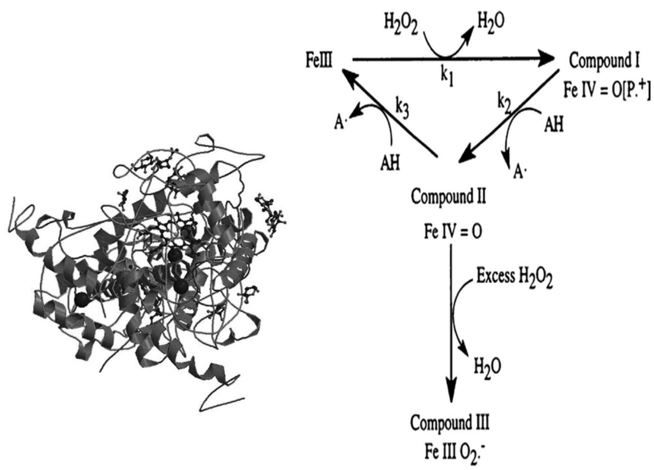
IFUS Point 4h: White Rot Fungi in cooperation with Brown Rot Fungi and a group of bacteria are all naturally found in Sugarcane Bagasse. Specifically, Hatakka showed that, "Basidiomycota, ligninolytic enzymes have been detected, prime examples being Agaricus bisporus producing laccase and MnP and Marasmius quercophilus producing only laccase (Hatakka A. Biodegradation of lignin. Alexander S, (ed.) Biopolymers Online, Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA, 2005).

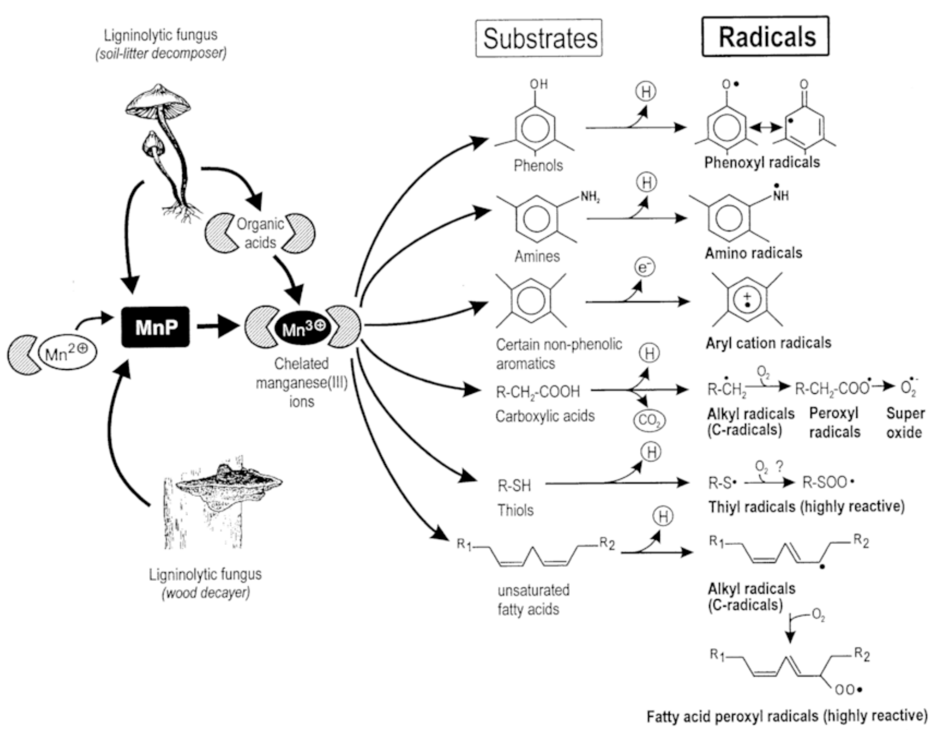
IFUS Point 4h(1): Each of these microbial groups contribute to specific biochemical processes shown to naturally degrade and depolymerize the lignin in Sugarcane Bagasse, yet increased the viability of several fungi.

IFUS Point 4i: Furthermore, White Rot Fungi, Brown Rot Fungi, and other microorganisms produce Cytochromes P450. "Cytochromes P450 (P450s or CYPs) are a superfamily of enzymes containing heme as a cofactor that mostly, but not exclusively, function as monooxygenases.” (Cytochrome P450. InterPro.)

IFUS Point 4(j): One example of lignin depolymerization. “The O-demethylation of lignin aromatics is a rate-limiting step in their bioconversion to higher-value compounds. A recently discovered cytochrome P450, SyoA, demethylates the S-lignin aromatic syringol.” (Harlington, A.C., et al., Structural insights into S-lignin O-demethylation via a rare class of heme peroxygenase enzymes. Nat Commun 16, 1815(2025). https://doi.org/10.1038/s41467-025-57129-6

IFUS Point 4j(1): A reminder: Sugarcane Bagasse contains 60% S-lignin, plus Mn and Fe critical to heme production. (IFUS Point 4g & Diagrams Below):





IFUS Point 4k: There are volumes of studies in support of the depolymerization of lignin by White Rot Fungi, Brown Rot Fungi, and various bacteria naturally found in Sugarcane Bagasse. As another example, “Metabolic mechanism of lignin-derived aromatics in white-rot fungi,” Hiroyuki Kato, et.al., Appl Microbiol Biotechnol. 2024 Dec 11;108(1):532. doi: 10.1007/s00253-024-13371-4.

IFUS Point 4l: “United States Patent Office 3,597,218 METHOD FOR PREPARATION OF FEED Seiko Matsuoka, 9-30 Yanagi-machi, Nase-shi, Kagoshima-ken, Japan No Drawing. Filed Sept. 4, 1968, Ser. No. 757,501 Claims priority, application Japan, Sept. 6, 1967, 42/ 57,671 Int. Cl. A23k U5. Cl. 99-9 3 Claims ABSTRACT OF THE DISCLOSURE A method of preparing an animal feed from bagasse which comprises culturing a cellulose-decomposing microorganism, a nitrate-forming microorganism, a starch hydrolyzing and proteolytic microorganism, and a lignin decomposing microorganism together in a medium consisting of wheat bran and rice bran to obtain a composite inoculum, inoculating bagasse with said inoculum and culturing it for 24 hours. BACKGROUND OF THE INVENTION: This invention relates to a method of preparing a feed having high digestibility from undigestible bagasse (sugar cane from which sugar juice has been extracted) containing cellulose and lignin by applying an enzyme which decomposes cellulose and lignin.”

IFUS Point 4m: Furthermore, another study shows, “Microbial degradation of lignin: Role of lignin peroxidase, manganese peroxidase, and laccase,” Takayoshi Higuchi, Proc Jpn Acad Ser B Phys Biol Sci. 2004 May 1;80(5):204–214. “Lignin peroxidase (LiP), laccase (LA) and manganese peroxidase (MnP) of white-rot basidiomycetes such as Phanerochaete chrysosporium, Coliorus versicolor, Phlebia radiata and Pleurotus eryngii catalyze oxidative degradation of lignin substructure model compounds and synthetic lignins (DHPs). Thus, the role of LiP, LA, and probably MnP in lignin biodegradation could be explained by the following unifying view”:

“Enzymatic reaction

1. LiP/ H_2O_2 → Phenoxy radicals of phenolic units, and aryl cation radicals or cation radicals of non-phenolic units

2. LA/O_2 → Phenoxy radicals of phenolic units

3. LA/O_2+Mediators → Phenoxy radicals of phenolic units, and aryl cation radicals or cation radicals of nonphenolic units

4. MnP/H_2O_2+Mn^{2+} → Phenoxy radicals of phenolic units

5. MnP/H_2O_2+Mn^{2+}+Mediators → Phenoxy radicals of phenolic units and aryl cation radicals or cation radicals of non-phenolic units

Non-enzymatic reaction

1. Homolytic or heterolytic cleavage of side chains (C$\boldsymbol{\alpha}$-C$\boldsymbol{\beta}$, alkyl-phenyl), and aromatic rings

2. O_2 attack on carbon-centered radical intermediates

3. Nucleophilic attack on aryl cations and C$\boldsymbol{\alpha}$ cations by H_2O and R-OH → Degradation products"

IFUS Point 5: Hence, what does the Chemistry (Organic/Inorganic/Physical), Biochemistry, Polymer Chemistry, Thermodynamics/Kinetics, Virology, Bacteriology, Mycology, and a dash of Geology/Geophysics tell us about "Degraded", "Depolymerized", and intact Lignin as it relates to Bovine Herd Performance. Based in these scientific disciplines, one would wonder whether Forage Analysis or Manure Analysis possess the capability in isolation to demonstrate the efficacy of SGP+™, especially in consideration of the required C-Change in thinking if Bovine Ration Management is to address present challenges affecting the beef and dairy industries. So to the question of Crude Protein:

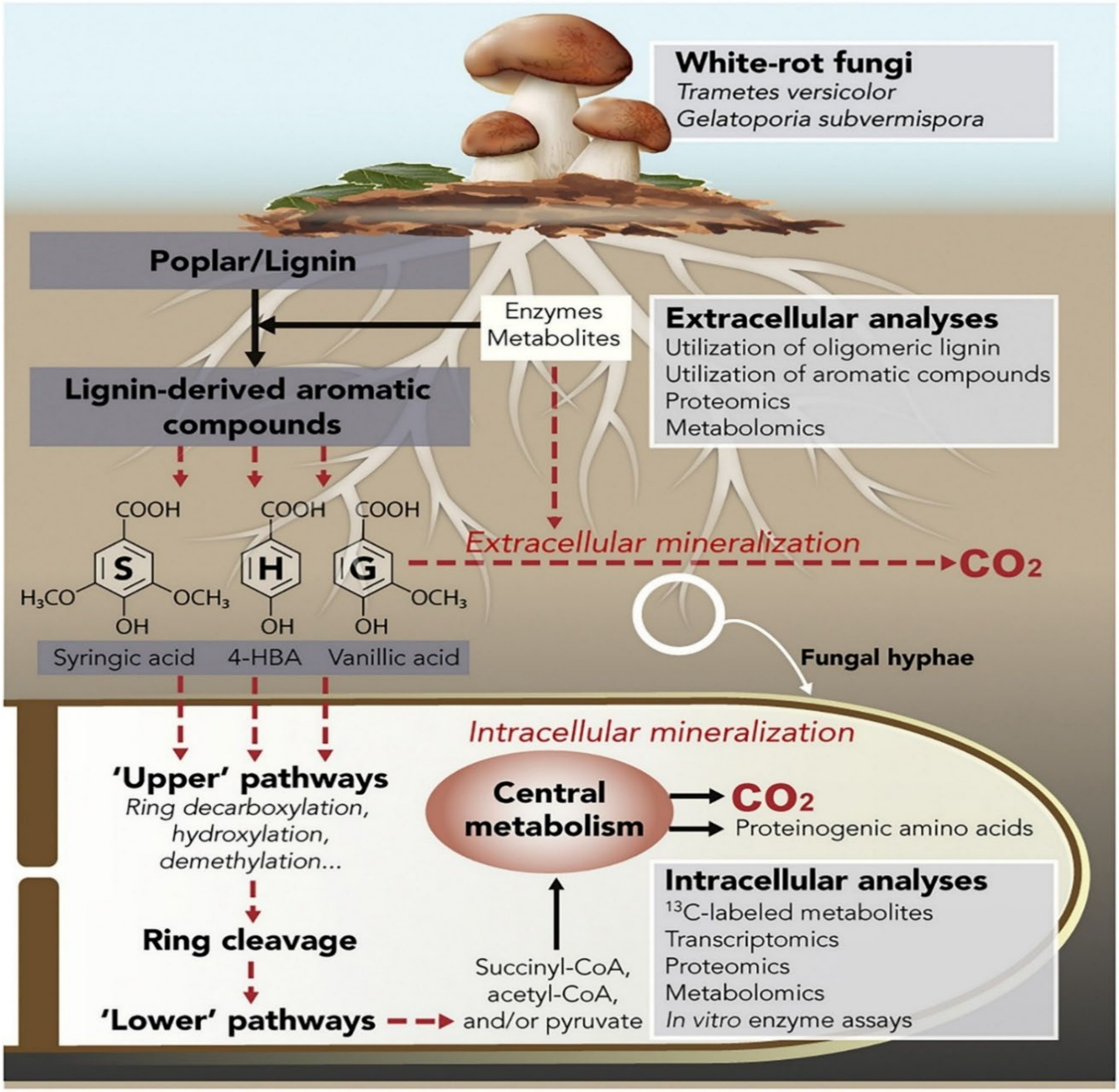
IFUS Point 5a: **CRUDE PROTEIN FROM LIGNIN:** "The fungus Pleurotus ostreatus NRRL-2366 degraded 56.7% and 45.9% of untreated and chemically pretreated (delignified) sugarcane bagasse, respectively, during 14-day incubation in a submerged fermentation process. The biodegradation percentages of cellulose, hemicellulose and lignin were 33.0%, 72.5% and 14.5%, respectively. An increment of 22.6% of crude protein content in the residual fermented material was observed. Chemical composition of the end-product and its amino acids profile were reported." "Bioconversion of sugarcane bagasse into a protein-rich product by white rot fungus", Samir A. El-Sayed, et.al., Resources, Conservation and Recycling, Volume 12, Issues 3–4, November 1994, Pages 195-200 (https://doi.org/10.1016/0921-3449(94)90007-8).

IFUS Point 5a(1): The science is suggesting to us that degradation and some depolymerization of lignin begins *in vitro*. However, further science is suggesting to us that the actual value-added depolymerization is occurring in the bovine's digestive system (or *in vivo*). Assuming there is merit to this thinking, then present Forage Analysis and even Manure Analysis in isolation would not be designed to determine the actual protein efficacy of SGP+™, especially in consideration a shift in Nitrogen generation, processing, and reclamation cycles *in vivo*. A series of studies further elucidate this thinking.

IFUS Point 5a(2): "The lignin-degrading microorganisms of white-rot fungi, brown-rot fungi, soft-rot fungi, and bacteria under aerobic and anaerobic conditions were comparatively analyzed. The catalytic metabolism of the microbial lignin-degrading enzymes of laccase, lignin peroxidase, manganese peroxidase, biphenyl bond cleavage enzyme, versatile peroxidase, and β-etherize was discussed. The microbial metabolic process of H-lignin, G-lignin, S-lignin based derivatives, protocatechuic acid, and catechol was reviewed. Lignin was depolymerized to lignin-derived aromatic compounds by the secreted enzymes of fungi and bacteria, and the aromatics were converted to value-added compounds through microbial catalysis and metabolic engineering. "Depolymerization and conversion of lignin to value-added bioproducts by microbial and enzymatic catalysis," Caihong Weng, et.al., Biotechnol Biofuels. 2021 Apr 3;14:84. doi: 10.1186/s13068-021-01934-w

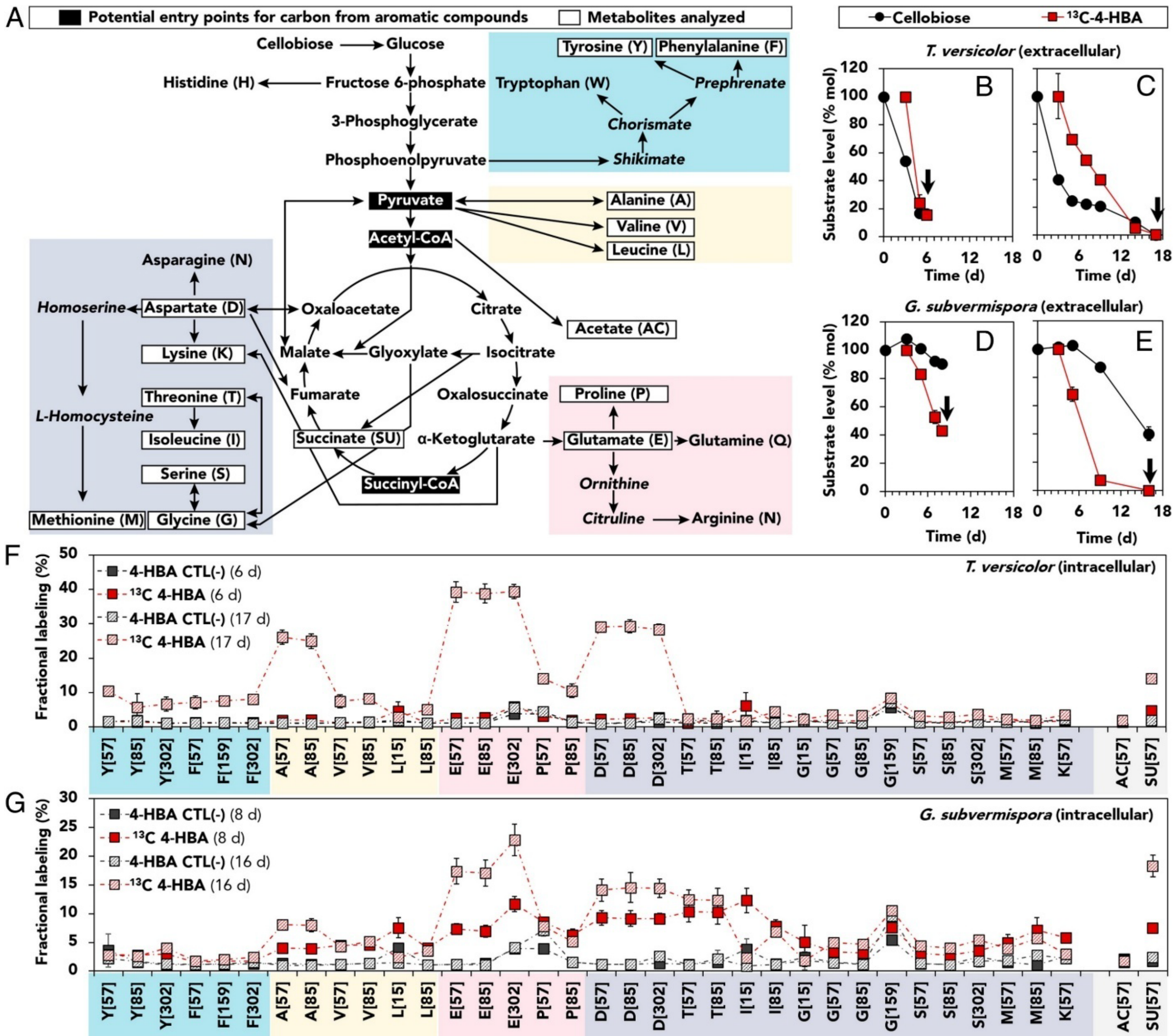
IFUS Point5a(3): Furthermore, where does the protein come from? An answer has been provided by a recent breakthrough whereby Proteinogenic Amino Acids are produced in the presence of minerals from lignin depolymerization. Source: "Intracellular pathways for lignin catabolism in white-rot fungi", Carlos del Cerro, et.al, The Proceedings of the National Academy of Sciences: Systems Biology/Biological Sciences, Feb. 23, 2021.

This is illustrated in the diagram below on work done on Poplar Lignin. However, the pathways of S-, G-, and H-Lignin are shown to produce Proteinogenic Amino Acids (See Next Page):



IFUS Point 5a(4): Among the Amino Acids produced, Lysine and several other amino acids critical to bovine colostrum/milk production are produced. (See: "Amino Acid Production from Lignin by White Rot Fungus Fig. 2") https://www.pnas.org/doi/10.1073/pnas.2017381118#fig02, "Intracellular pathways for lignin catabolism in white-rot fungi", Carlos del Cerro, Erika Erickson, Tao Dong, Davinia Salvachúa, et.al, The Proceedings of the National Academy of Sciences: Systems Biology/Biological Sciences, Feb. 23, 2021.

Fig. 2 below reinforces this science:



C-labeling experiments demonstrate carbon flux of lignin-derived compounds to central metabolism. (A) Abbreviated map showing central carbon metabolic pathways and amino acid biosynthesis in WRF based on the current KEGG model for T. versicolor. (B–E) Substrate level (molar percent) of extracellular metabolites (cellobiose and 4-HBA) in (B) 6-d and (C) 17-d T. versicolor cultivations and (D) 8-d and (E) 16-d G. subvermispora cultivations. A value of 100% corresponds to the initial concentration (7.5 mM in all cases, excluding cellobiose concentration in the 17-d T. versicolor cultivations, which was 3.75 mM). Arrows indicate the sample collection time for 13C intracellular analysis. (F–G) Fractional labeling in detected proteinogenic amino acid fragments and other metabolites (acetate and succinate) in

(F) T. versicolor and (G) G. subvermispora cultivations when providing unlabeled 4-HBA [negative control, CTL(-)] and 13C-ring–labeled 4-HBA. Amino acids fragments (i.e., [15], [57], [85], [159], and [302]) are the result of the derivatization and analysis as thoroughly detailed by Nalsen et al. (33). Individual points are connected with discontinuous lines to facilitate visualization. All results are the average of biological triplicates, and error bars represent the SD. Statistical significance (t test) is presented in SI Appendix.

IFUS Point 5a(5): The biochemistry is telling us that the chemical and enzymatic secretions within the bovine digestive system are now capable of digesting the degraded and somewhat depolymerized lignin, more than ever in the Upper GI. "Highly Promiscuous Oxidases Discovered in the Bovine Rumen Microbiome," Lisa Ufarté, et.al., Microbiol. 2018 May 4;9:861. doi: 10.3389/fmicb.2018.00861. Furthermore, IFUS Points 5b-5f attempt to explain the synergies in the biochemistry that supports this IFUS Point.

IFUS Point 5a(6): An additional study adds further credibility to this line of thinking: "Comprehensive assessment of the L-lysine production process from fermentation of sugarcane molasses," Omar Anaya-Reza, et.al., Bioprocess Biosyst Eng. 2017 Jul;40(7):1033-1048. doi: 10.1007/s00449-017-1766-2. Epub 2017 Apr 13. (PMID: 28409400 DOI: 10.1007/s00449-017-1766-2)

IFUS Point 5b: **Degraded / Depolymerized Lignin Produces a Natural Estrogenic Precursor System**, critical to lactation, heifer bone-structure, and uterine development.

IFUS Point 5b(1): "Lignin enhances the degradation of 17β-estradiol (E2) in bovine milk production. E2 has been shown to decrease milk production in dairy cows during gestation. The presence of 17β-oestradiol, estrone, and estriol-sulphate in human breast cyst fluid has also been demonstrated." "The role of lignin in 17β-estradiol biodegradation: insights from cellular characteristics and lipidomics," Hanyu Pan, et.al., Microb Cell Fact 23, 347 (2024). https://doi.org/10.1186/s12934-024-02605-9

IFUS Point 5b(2): "Abstract: Estradiol inhibits milk production in dairy cows." "Effect of 17β-estradiol on milk production, hormone secretion, and mammary gland gene expression in dairy cows," J.J. Tong, I.M. Thompson, X. Zhao, P. Lacasse, "Effect of 17β-estradiol on milk production, hormone secretion, and mammary gland gene expression in dairy cows," Journal of Dairy Science Volume 101, Issue 3, March 2018, Pages 2588-2601

IFUS Point 5b(3): "Degraded lignin can be supportive for natural phytoestrogens in bovines (1)(2)(3)(4)(5). In nature, lignin can be degraded by microorganisms and their secreted enzymes into building blocks that can potentially be converted into high-value chemicals (1). Microbial degradation of lignin by fungi, important lignin degraders in nature, has been intensively studied."

A series of studies support the thinking of natural estrogen and/or estrogen precursors:

(1) Ref.1: "Biological degradation of lignin: A critical review on progress and perspectives," Lei Zhao, et.al., Industrial Crops and Products Volume 188, Part B, 15 November 2022, 115715

(2) Ref.2: "Deconstruction of Lignin: From Enzymes to Microorganisms," Jéssica P Silva, et.al, Molecules. 2021 Apr 15;26(8):2299. doi: 10.3390/molecules26082299

(3) Ref.3: "Effects of purified lignin on in vitro rumen metabolism and growth performance of feedlot cattle," Yuxi Wang 1, et.al., Asian-Australas J Anim Sci, 2016 Jul 14;30(3):392–399. doi: 10.5713/ajas.16.0317

(4) Ref.4: "A trashed treasure: Lignin could become a large and renewable source of organic compounds for the chemical industry to replace fossil fuel-based chemicals," Maria Vigh, EMBO Rep. 2023 Mar 22;24(5):e57103. doi: 10.15252/embr.202357103

(5) Ref.5: "Highly Promiscuous Oxidases Discovered in the Bovine Rumen Microbiome," Lisa Ufarté, et.al., Front Microbiol. 2018 May 4;9:861. doi: 10.3389/fmicb.2018.00861.

IFUS Point 5c: **Degraded/Depolymerized Lignin, Carob, and Mastic Produce Natural Tannins Known to Improve Bovine Hide-quality.** "Bovine hide collagen/tannin extract composite: A revelation of the selective structure-activity relationship between phenolic hydroxyls and Cu(II) and study on adsorption properties," Feng Zhao, et.al., "Colloids and Surfaces A: Physicochemical and Engineering Aspects", Volume 682, 5 February 2024, 132886.

IFUS Point 5c(1): "Carob contains tannins, which act as antioxidants without observed toxicity and contribute to its mild astringency. The aqueous extract of carob leaves is rich in phenolic compounds, including flavonoids and hydrolyzable tannins. The primary mechanism mainly involves the production of nitric oxide by the endothelium via the Ca2+-calmodulin-NOS complex. "Carob leaves: Phytochemistry, antioxidant properties, vasorelaxant effect and mechanism of action," Widad Dahmani, et.al., Journal of Ethnopharmacology, Volume 340, 31 January 2025, 119226

IFUS Point 5c(2): Various biochemical pathways demonstrate that each of these compounds play an interactive role in SGP+™. Of particular interest are those compounds that effect bovine hide-quality. See Table 1: "Functional Components of Carob Fruit: Linking the Chemical and Biological Space," Vlasios Goulas, et.al., Int J Mol Sci. 2016 Nov 10;17(11):1875. doi: 10.3390/ijms17111875

Table 1.

A summary of most common phenolic compounds in different parts of carob fruit.

Polyphenol	Carob Part/Fraction	Reference
Phenolic acids		
4-hydroxybenzoic acid	Pulp	[57]

Polyphenol	Carob Part/Fraction	Reference
Caffeic acid	Pulp	[57]
Chlorogenic acid	Seed	[60]
Cinnamic acid	Fiber, pulp	[57,61]
Coumaric acid	Fiber, pulp	[57,61]
Ferulic acid	Fiber, pulp, seed	[57,60,61]
Gallic acid	Pulp, fiber, seed	[53,60,62]
Gentisic acid	Seed	[60]
Syringic acid	Pulp, seed	[57,60]
Flavonoids		
(epi)gallocatechin	Fiber	[53]
(epi)gallocatechingallate	Fiber	[53]
Apigenin	Fiber, pulp	[57,61]
Catechin	Pulp, seed	[53,60,62]
Chrysoeriol	Fiber, pulp	[57,61]
Eridictyol	Pulp	[57]

Polyphenol	Carob Part/Fraction	Reference
Genistein	Pulp	[57]
Isorhamnetin	Fiber, pulp	[57,61]
Kaempferol	Fiber, pulp	[53,57,61]
Kaempferol rhamnoside	Fiber	[61]
Kaempferol-desoxyhexoside and -dihexoside	Pulp	[53]
Luteolin	Fiber, pulp	[57,61]
Myricetin	Seed	[60]
Myricetin rhamnoside and -desoxyhexoside	Fiber	[61]
Myricetin-hexoside	Fiber, pulp	[53,57,61]
Naringenin	Fiber, pulp	[57,61]
Quercetin	Fiber, seed	[60,61]
Quercetin-arabinoside	Fiber	[61]
Quercetin-desoxyhexoside and -hexoside	Fiber, pulp	[53]
Quercetin rhamnoside	Pulp	[57]

Polyphenol	Carob Part/Fraction	Reference
Tricetin 3′, 5′ dimethyl ether	Fiber, pulp	[57,61]
Tannins		
(epi)gallocatechin + 4 gallic acid units	Fiber	[53]
Hexose + 2 or 3 or 4 or 5 Gallic acid Units	Fiber	[53,61]
Pentoses + 2 gallic acid units	Fiber	[53]
prodelphinidin dimer and trimer	Fiber	[53]

IFUS Point 5c(3): Furthermore, when overlayed, the synergies of Mastic, Carob, Ionic Minerals, Water, and Lignin (plus other components of Sugarcane Bagasse) appear more than "coincidental."

IFUS Point 5d: **DEGRADED / DEPOLYMERIZED LIGNIN ALSO PRODUCES TANNINS KNOWN TO REPEL FLIES / INSECTS:**

IFUS Point 5d(1): Of interest, not a single insect can be found inside a Raw Sugar warehouse, nor on a Sugarcane Bagasse pile. It is not until the raw sugar is dissolved with water that any bee will be attracted to it.

However, flies are not attracted to raw sugar even when it dissolves.

IFUS Point 5d(2): Where SGP+™ is stored, no insects can be found, even though when fresh product is produced and stored, the warehouse smells like a Starbucks coffee shop.

IFUS Point 5d(3): "Research shows that tannins can help control internal parasites in livestock. By affecting the gut environment, they create conditions less favorable for parasite survival and reproduction. This effect can lead to healthier animals with improved growth performance." "Tannin in Ruminant Nutrition: Review", Maghsoud Besharati, et.al., Molecules. 2022 Nov 27;27(23):8273. doi: 10.3390/molecules27238273 , and "The effects of tannin-rich plants on parasitic nematodes in ruminants," Hervé Hoste, et.al., "The effects of tannin-rich plants on parasitic nematodes in ruminants," Trends Parasitol. 2006 Jun;22(6):253-61. doi: 10.1016/j.pt.2006.04.004.

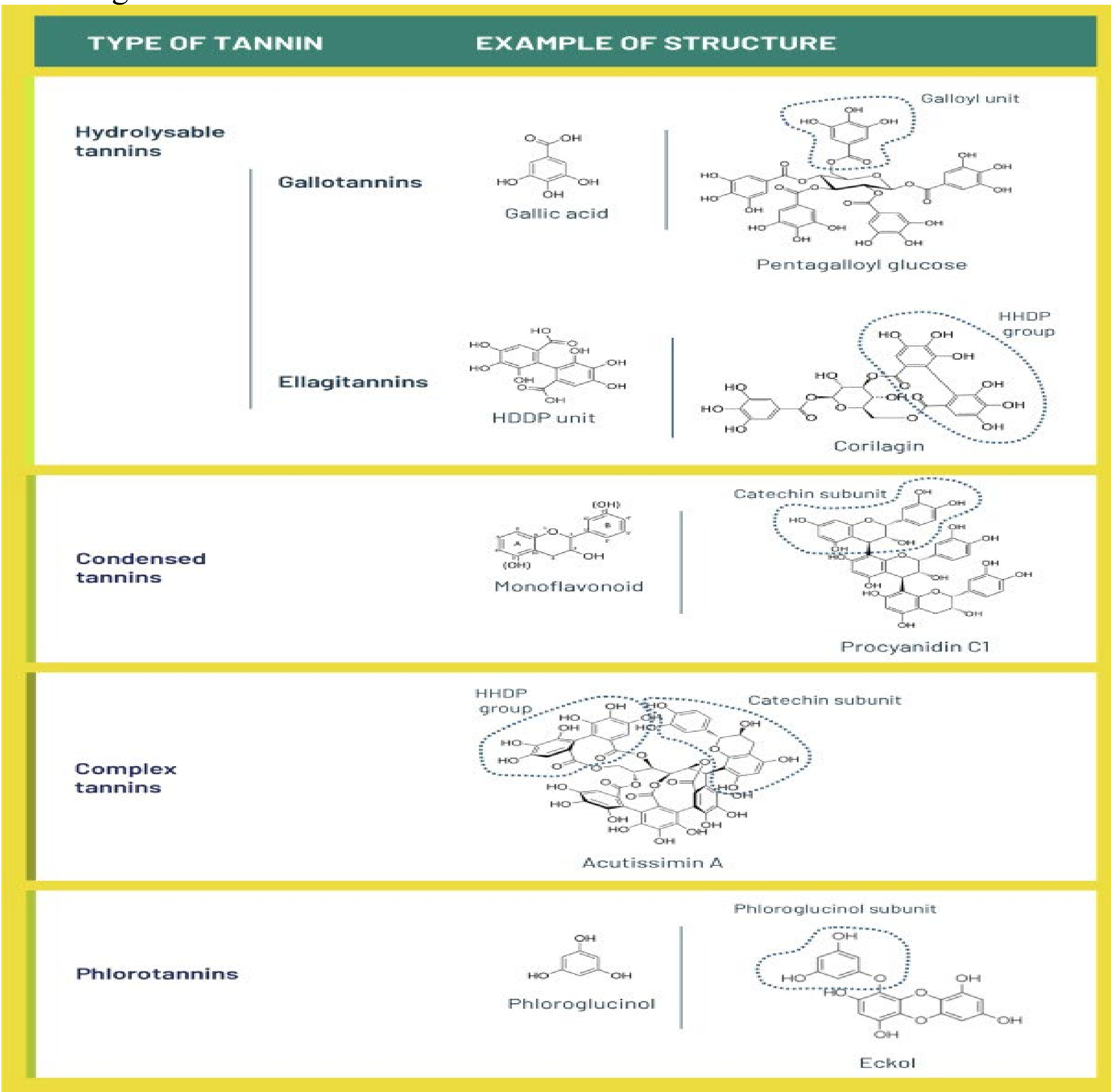
IFUS Point 5d(4): A cow hide contains collagen. "Collagen can absorb plant tannins, and the interaction between plant tannin and hide collagen has been investigated to clarify the cross-linking mechanism. The primary reaction is through multiple hydrogen bonding, while the subsequent reaction is to crosslink the tannin molecules together, creating matrices of crosslinked polyphenolic species bound to collagen effectively working as a single moiety." "Investigations Towards the Binding Mechanisms of Vegetable Tanning Agents to Collagen," Michaela Schröpfer, et.al., Research Journal of Phytochemistry, June 2016 (10(2):58-66, DOI:10.3923/rjphyto.2016.58.66)

IFUS Point 5d(5); Mastic and Carob both contain Gallic Acid. Gallic Acid is part of the family of Gallotannins.

IFUS Point 5d(6): "Gallotannins are scarcely found in nature and are the most basic hydrolysable tannins, consisting of gallic acid derivatives which contain six or more galloyl groups (Fig. 2)."

Fig. 2. Structures of tannins. "Even though the described compounds are key intermediates in the biosynthesis of nearly all hydrolysable plant polyphenols, it is quite infrequent to find gallotannins in nature compared to ellagitannins. However, gallotannins, especially hexa to decagalloyl glucoses, are exploited extensively at industrial level as commercially available tannic acid, and principally used for protein precipitation (Salminen & Karonen, 2011)."

Fig.2:



IFUS Point 5d(7): "Gallotannins are tannins that contain galloyl or diverse polyol-, catechin-, or triterpenoid units. They are the hydrostable and simplest form of tannins, characterized by a polyphenolic and a polyol residue, with variations in the polyol residue." "Chapter: Glucose Transporter 4 Translocation Activators From Nature," K. Dev, et.al., Discovery and Development of Antidiabetic Agents from Natural Products. Natural Product Drug Discovery, A volume in Natural Product Drug Discovery, 2017.

IFUS Point 5d(8): “Gallotannins, a class of tannins, commonly occur in many plant species and cause insect toxicity. They are potent inhibitors and disruptors of bacteria2. While they don't fully repel bugs, they can partially repel insects.” “Recognition of Gallotannins and the Physiological Activities: From Chemical View,” Hua-Feng He, Front Nutr. 2022 Jun 1;9:888892. doi: [10.3389/fnut.2022.888892](https://doi.org/10.3389/fnut.2022.888892)

IFUS Point 5d(9): “Gallotannins, characterized with the glycosidic core and galloyl unit, are seemed as vital component of hydrolyzable tannins. More than that, activities involving in antioxidant, anti-inflammatory, enzyme inhibitions, protein binding, and so on, as well as applications in the field of food industry, biopharmaceutical science, agricultural production, etc., were combed.”

IFUS Point 5d(10): Furthermore, “Mastic contains triterpenoids, which are found in plants, and repel insects. They mediate plant-insect interactions, working to the benefit of the plants and to the detriment of herbivores. These terpenoids have insecticidal potential and can act as contact poisons, stomach poisons, antifeedants, or growth inhibitors on various agricultural insects.

IFUS Point 5d(11): “Carob also contains a panel of polyphenols; gallic acid and its derivatives, as well as condensed tannins are the most representative phenolic compounds in carob fruit. The presence of some minerals and amino acids are also important for human health, so its implementation in a daily diet can help reduce incidents such as heart diseases ...” “Functional Components of Carob Fruit: Linking the Chemical and Biological Space,” Vlasios Goulas, et.al., Int J Mol Sci. 2016 Nov 10;17(11):1875. doi: 10.3390/ijms17111875.

IFUS Point 5d(12): “The sweet pods, high in fibre are eaten by horses, cattle, pigs, goats and rabbits. Do not feed to chickens. Whole pods (and very hard seeds) should be crushed in a hammermill. Carob seeds are extremely hard and unless ground before feeding, may pass undigested through the animal’s digestive tract. Carob seeds contain about 18% protein and 42 - 46% gum. Carob is high in tannins and should not make up more than 10% of an animal feed. These tannins interfere with protein digestibility. Carob seed flour is high in protein and is used in cattle feed as well as in dog biscuits. Carob flour dissolves easily in water and can be used as a weaning diet for piglets, calves and other ruminants. Some farmers feed carob to their horses as part of a ‘cool feed’ (high fibre) or to replace sugary treats such as liquorice. The pods are ground in a hammermill, but some horses eat the pods directly from the ground. Carob as rabbit feed was tested in an Egyptian study and it was found that including 5% of carob pods in the diet stimulated the performance of growing rabbits." Carob as Animal Feed, Indigenous Vegetables of South Africa, https://southafrica.co.za/carob-as-animal-feed.html.

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Unripe carob pods are green. The brown pods are harvested onto a net or canvas on the ground.

IFUS Point 5d(13): Microbes found in Sugarcane Bagasse can convert lignin into Gallotannins. "In the early 1960s, enzyme studies increasingly began to replace the common 'feeding' experiments in which labeled tracers were applied to living plants or plant parts for elucidating metabolic pathways. This advanced technique allowed to gain much deeper insights into individual details of metabolic sequences, and particularly on the previously inaccessible role of activated 'energy-rich' intermediates. Based on the author's own experience for the past 40+ years in this field, principal findings and trends elucidating the pathways to lignin and lignin precursors, acyl amides and hydrolyzable tannins (gallotannins, ellagitannins) by enzyme studies are reported. "From lignins to tannins: Forty years of enzyme studies on the biosynthesis of phenolic compounds," Georg G. Gross, Phytochemistry, Volume 69, Issue 18, December 2008, Pages 3018-3031.

IFUS Point 5d(14): Additionally, science tells us that furfurals produced in lower GI attract insects like flies, while flavonoids repel flies. Flavonoids are linked to Pheromones from tannic substances. "Flavonoids can be produced from lignin. Effective lignin depolymerization produces aromatic precursors for flavonoids, and the bioconversion of lignin derivatives into flavonoids holds potential for both lignin valorization and flavonoid synthesis." "Biological valorization of lignin to flavonoids," Hai-Na Lan, et.al., Biotechnology Advances, Volume 64, May–June 2023, 108107 and “Harnessing Aromatic Properties for Sustainable Bio-valorization of Lignin Derivatives into Flavonoids.” Si-Yu Zhu, et.al., Green Carbon, 2025.

IFUS Point 5d(15): “Lignin is first depolymerized via microorganisms and funneling various lignin-derived aromatics into protocatechuate and catechol.” Depolymerization and conversion of lignin to value-added bioproducts by microbial and enzymatic catalysis, Caihong Weng, et.al., Biotechnol Biofuels 14, 84 (2021). https://doi.org/10.1186/s13068-021-01934-w.

IFUS Point 5d(15a): “Approximately 50% of the synthetic catechol is consumed in the production of pesticides, the remainder being used as a precursor to fine chemicals such as perfumes and pharmaceuticals.” Fiege, Helmut; Voges, Heinz-Werner; Hamamoto, Toshikazu; Umemura, Sumio; Iwata, Tadao; Miki, Hisaya; Fujita, Yasuhiro; Buysch, Hans-Josef; Garbe, Dorothea; Paulus, Wilfried (2000), "Phenol Derivatives", Ullmann's Encyclopedia of Industrial Chemistry, doi:10.1002/14356007.a19_313, ISBN 978-3-527-30385-4

IFUS Point 5d(16): Joe Wilcox also reports a difference in his feedlot cows fed SGP+™ vs. his pasture cows not fed regular SGP+™. “Myself have experienced reduction of lice. Feedlot cows very little lice. Pasture cows - not fed as often have lice.”

IFUS Point 5e: **LIGNIN DEGRADATION/DEPOLYMERIZATION PRODUCES H2O** from (1) suppression of methanogenesis and (2) a separate acid/base reaction individually and possibly collectively resulting in quicker satiation of cattle (chewing cuds 25% quicker in morning and evening feedings), while reducing Greenhouse Gases though *in vivo* reduction of methanogenesis, accordingly.

IFUS Point 5e(1): “Lignin degradation and depolymerization from White Rot Fungi biochemically shown to produce water. H2 necessary for the production of CH4 in protein metabolism, is converted in water.” “Methane production through anaerobic digestion: Participation and digestion characteristics of cellulose, hemicellulose and lignin," Wanwu Li, Habiba Khalid, Zhe Zhu, Ruihong Zhang, Guangqing Liu, Chang Chen, Eva Thorin, "Applied Energy," Volume 226, 15 September 2018, Pages 1219-1228.

IFUS Point 5e(2): This claim is supported by ranchers feeding SGP+™ at 80% of total ration reporting: (1) Reduced hydration requirements, (2) Improved heat tolerance, (3) Reduced urinary output, (4) Reduced NH4 smell, and (5) Improved overall herd performance. Joe Wilcox, a rancher who is also a degreed Animal Scientist out Oklahoma State has calculated that based on his herds reduced water requirements and better heat tolerance that, "Of the 8.55 MM beef cattle on feed in the states which are on the Ogallala aquifer, when fed an 80% SGP+ ration year round, saves 16% of the water it takes to produce 20# of corn per day!"

IFUS Point 5e(3): "Methanogenesis, the microbial methane (CH4) production, is traditionally thought to anchor the mineralization of organic matter as the ultimate respiratory process in deep sediments, despite the presence of oxidized mineral phases, such as iron oxides. This process is carried out by archaea that have also been shown to be capable of reducing iron in high levels of electron donors such as hydrogen. The current pure culture study demonstrates that methanogenic archaea (Methanosarcina barkeri) rapidly switch from methanogenesis to iron-oxide reduction close to natural conditions, with nitrogen atmosphere, even when faced with substrate limitations. Intensive, biotic iron reduction was observed following the addition of poorly crystalline ferrihydrite and complex organic matter and was accompanied by inhibition of methane production." "Methanogens rapidly transition from methane production to iron reduction," O. Sivan, Geobiology, Volume14, Issue2, March 2016, Pages 190-203

IFUS Point 5e(4): "For carbohydrate metabolism to continue, reductants must be re-oxidized mainly through 4 disposal pathways, which include amination through incorporation of ammonia-N into the carbon chain for microbial amino acids synthesis; propionate synthesis through randomizing pathway via reducing oxaloacetate to malate and fumarate to succinate; propionate synthesis through non-randomizing pathway via lactate as an intermediate; and butyrate synthesis via Crotonoyl-CoA as precursor (Li et al., 2024). The unused reductants are further re-oxidized via H2-evolving hydrogenases to produce H2. Sufficient methane mitigation should be associated with increasing reductant disposal or electron incorporation for host-benefit metabolite synthesis, leading to a reduction in H2 and methane production." "Mitigating enteric methane emissions: An overview of methanogenesis, inhibitors and future prospects," "Mitigating enteric methane emissions: An overview of methanogenesis, inhibitors and future prospects," Xin Xie, et.al., Animal Nutrition Journal, 4Feb2025, https://doi.org/10.1016/j.aninu.2025.02.00.

IFUS Point 5e(5): Furthermore, if in fact CO2 is produced from lignin depolymerization in the bovine digestive system (from a reaction that converts the H2 into H20 so as to prevent methanogenesis), Henry's Law suggests that the pressure within the bovine rumen is sufficient enough to solubilize the CO2 into Carbonic Acid (a weak acid in the presence of HCl, hence a Lewis Base of sorts), and in the presence of Na+ or Ca+ is also able to form Sodium Bicarbonate and Calcium Bicarbonate, respectively.

IFUS Point 5e(6): "The answer came by changing my focus on a forgotten component of the ruminal buffering system, **dissolved CO2,** the primary acid in the rumen's buffering system (Turner and Hodgetts, 1955; Laporte-Uribe, 2016), Figure 1. The addition of molasses altered the rumen fluid's physicochemical properties, increasing its viscosity and hindering dCO2 release (effervescence). This phenomenon is known as CO2 holdup. As dCO2 formation rises and bicarbonate declines, ruminal pH falls, the Henderson-Hasselbalch equation (Table 1). "Ruminal CO2 Holdup: A New Perspective on Rumen Function," Dr. José A. Laporte Uribe, DVM, PhD Animal Science, Native Microbials, Jun 13, 2024.

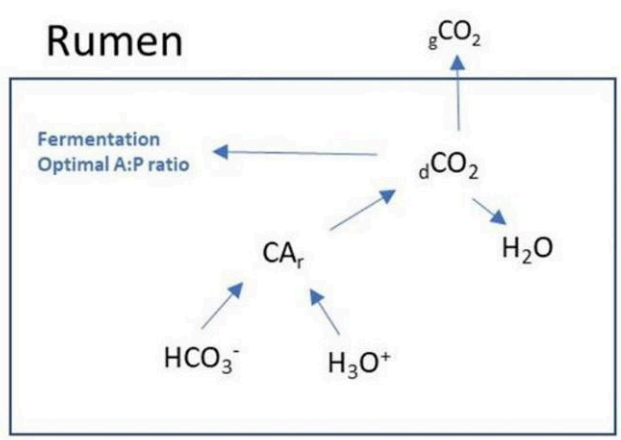


Figure 1. The ruminal buffering system: Protons (H_3O^+) are neutralized in the rumen by bicarbonate (HCO_3^-). This reaction is facilitated by ruminal carbonic anhydrase (enzyme). The process results in the formation of dissolved carbon dioxide (dCO_2). As water (H_2O) is released and CO_2 gas escapes to the gas cap, the system's buffering capacity is maintained. Remarkably, ruminal dCO_2 is the key component and its concentrations in the fluid promote optimal fermentation. This principle is similar to the fizz you see in carbonated beverages like beer or soda.

This phenomenon offers a crucial benefit. Increased dCO_2 availability provides beneficial bacteria with more substrate to produce lactate and succinate, ultimately leading to higher propionate production – the primary energy source for cattle milk yield (Aschenbach et al., 2010). Additionally, this favourable environment for propionate-producing bacteria leads to higher hydrogen retention, outcompeting methanogens and reducing methane emissions (Russell, 1998), Figure 1 (above).

IFUS Point 5e(7): Hence, if the average maximum capacity of the rumen = 100 liters. If the average rumen temperature is 39C with pH=6.8. Then PV=nRT suggests that the average Pressure inside the rumen = 3.76psi or 0.256atm. CO_2 solubility in water is considered to be 1-to-1. Hence, at 0.256atm with a 100-liter capacity, 0.256 liters of CO_2 are dissolved. Considering the dosage of Sodium Bicarbonate and/or Calcium Bicarbonate, one could take a SWAG of plausibility that buffering is occurring from CO_2 produced from lignin depolymerization (easing the digestive process) and explaining why ranchers and dairymen claim that their herds are burping less. However, just this week our IFUS Scientific Team came across this study: "Ruminal CO2 Holdup: A New Perspective on Rumen Function," Native Microbials, Jun 13, 2024. (And, the added CO_2 is being produced from the enhanced depolymerization of the lignin within in the rumen.)

IFUS Point 5e(8): This is notwithstanding the effect of Mastic and/or Carob on ruminal pH.

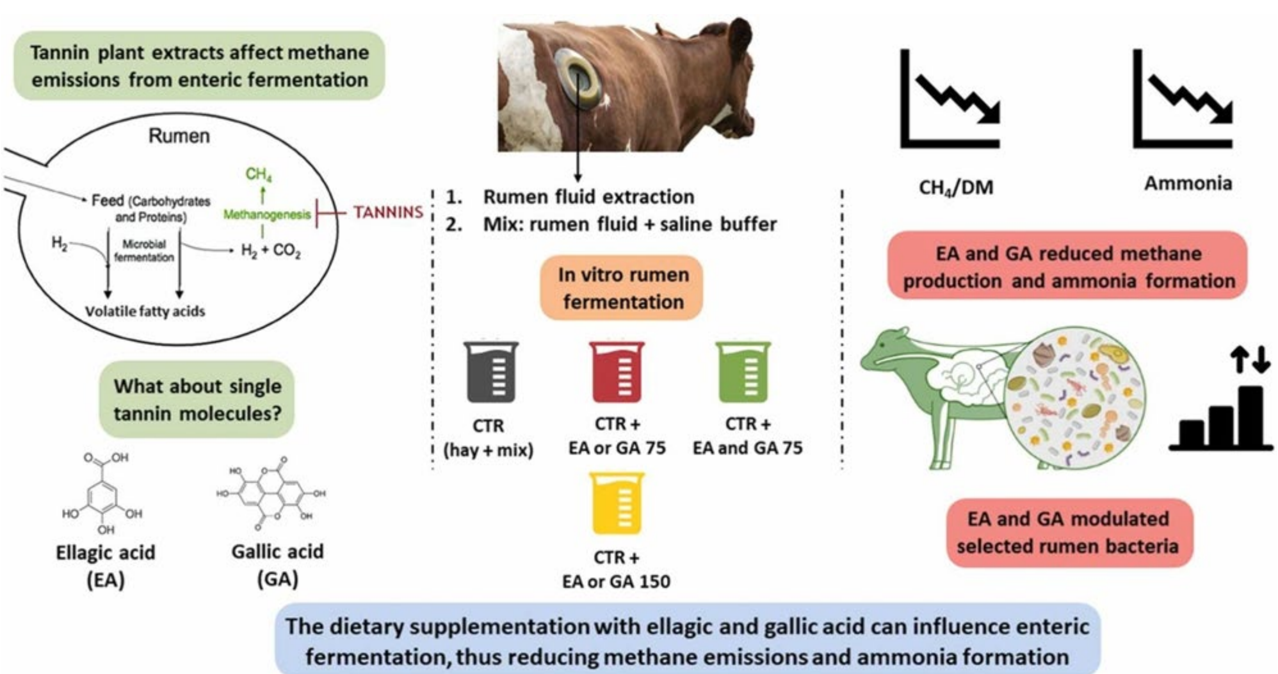
IFUS Point 5e(9); And this buffering decreases the HCl concentration in the rumen as less HCl is required due to the ease of digestibility of ration mixed with 80+% SGP+™ due to the depolymerization of the lignin (especially the S-lignin) making readily available energy and nutrition. Would this then become a true C-Change in Bovine Ration Management, especially if the secretion of NH4, HCl, and other digestive enzymes decreased?

IFUS Point 5f: **LIGNIN DEGRADATION / DEPOLYMERIZATION SHOWN TO SEQUESTER GREENHOUSE GASES**:

IFUS Point 5f(1): Gallic Acid is produced in lignin degradation and is an active ingredient in Chios Mastic Gum and Carob. Michele Manoni, et.al. in Animal Feed Science and Technology, Volume 305, November 2023, 115791, demonstrate the "Effect of ellagic and gallic acid on the mitigation of methane production and ammonia formation in an in vitro model of short-term rumen fermentation, Animal Feed Science and Technology, Volume 305, November 2023, 115791."

IFUS Point 5f(1a) "Highlights:

- Ellagic and gallic acid were assessed in an in vitro model of rumen fermentation.
- Ellagic and gallic acid decreased methane emissions and ammonia formation.
- Ellagic and gallic acid modulated the rumen microbial community."
- See Graphical Abstract below:



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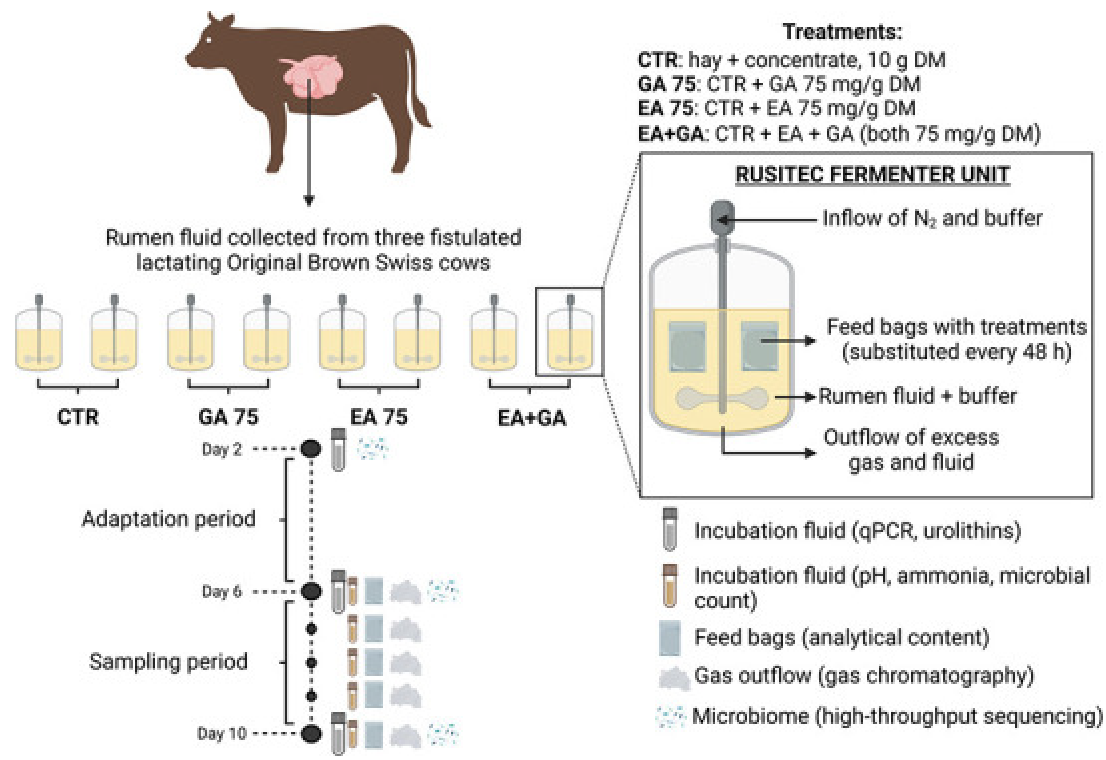
IFUS Point 5f(1b): From the information above, one could plausibly extrapolate that Ellagic Acid produced from microbial activity in Sugarcane Bagasse balances the Gallic Acid from SGP+™. The Ellagic Acid enhances bovine digestion and subsequent absorption, while the Gallic Acid suppresses methane, especially if the *in vitro* degradation and depolymerization of lignin continues *in vivo* in the Upper GI of the bovine (where the lignin degradation allows free H+ to be converted into water rather than CH4). (See IFUS Point 5f(2)). (Also, a Part 2 White Paper is under construction, which further explores the effects of Mastic Gum and Carob on Bovine Herd Performance, to include Greenhouse Gas sequestration.)

IFUS Point 5f(1b-1): One could view this as a progressive unraveling of lignin being sheared and shredded simultaneously with that progression beginning with the least cross-linked part of the polymer S-lignin and progressing to G-Lignin and then H-Lignin. The sequential nature of degradation followed by depolymerization makes each part of the polymeric chain more susceptible through surface area exposure, hence making the chain vulnerable to chemical interactions (enzymatic and other) through an increase in the Delta S (from a thermodynamic perspective).

IFUS Point 5f(1b-2): In a follow-up study, "Gallic and Ellagic Acids Differentially Affect Microbial Community Structures and Methane Emission When Using a Rumen Simulation Technique," Michele Manoni, et.al., J Agric Food Chem. 2024 Nov 26;72(49):27163–27176. doi: 10.1021/acs.jafc.4c06214, PMCID: PMC11638960 PMID: 39588639, it is revealed that:

"Dietary tannins can affect rumen microbiota and enteric fermentation to mitigate methane emissions, although such effects have not yet been fully elucidated. We tested two subunits of hydrolyzable tannins named gallic acid (GA) and ellagic acid (EA), alone (75 mg/g DM each) or combined (150 mg/g DM in total), using the Rusitec system. EA and EA+GA treatments decreased methane production, volatile fatty acids, nutrient degradation, relative abundance of Butyrivibrio fibrisolvens, Fibrobacter succinogenes, Ruminococcus flavefaciens but increased Selenomonas ruminantium. EA and EA+GA increased urolithins A and B. Also, EA and EA+GA reduced bacterial richness, with limited effects on archaeal richness. For bacteria, Megasphaera elsdenii was more abundant after EA and EA+GA, while Methanomethylophilaceae dominated archaea in all treatments. EA was more effective than GA in altering rumen microbiota and fermentation but GA did not reduce VFA and nutrient degradation. Thus, dietary supplementation of EA-plant extracts for ruminants may be considered to mitigate enteric methane, although a suitable dosage must be ensured to minimize the negative effects on fermentation."



IFUS Point 5f(1c): When overlayed with the claims being made by ranchers and dairymen as to Bovine Herd Performance when they apply SGP+™ as part of their respective Bovine Ration Management, then the extrapolation seems plausible and even possible. Especially when one considers that "Nineteen fungi and seven yeast strains were isolated from sugarcane bagasse piles from an alcohol plant located at Brazilian Cerrado and identified up to species level on the basis of the gene sequencing of 5.8S-ITS and 26S ribosomal DNA regions. Four species were identified: Kluyveromyces marxianus, Aspergillus niger, Aspergillus sydowii and Aspergillus fumigatus, and the isolates were screened for the production of key enzymes in the saccharification of lignocellulosic material. Among them, three strains were selected as good producers of hemicellulolitic enzymes: A. niger (SBCM3), A. sydowii (SBCM7) and A. fumigatus (SBC4). ("Thermotolerant and mesophylic fungi from sugarcane bagasse and their prospection for biomass-degrading enzyme production," Bruna Silveira Lamanes dos Santos, et.al., Braz J Microbiol. 2015 Jul 1;46(3):903–910. doi: 10.1590/S1517-838246320140393.

IFUS Point 5f(1d-1): Aspergillus niger "produces many useful enzymes for the catabolism of biopolymers in order to obtain nutrients from its environment.[30]." A. niger can produce "Alpha-galactosidase (GH27), an enzyme that breaks down certain complex sugars, is a component of Beano and several other products that decrease flatulence.[32]" ("Man…I'm tellin' ya…there ain't no smell in my pasture…and I swear our cows aren't fartin' as much," states a rancher in Jefferson, TX now on his 12th year of feeding his herd SGP+™. Furthermore, "Various strains of A. niger are used in the industrial preparation of citric acid (E330) and gluconic acid (E574); therefore, they have been deemed acceptable for daily intake by the World Health Organization.[24] A. niger fermentation is "generally recognized as safe" (GRAS) by the United States Food and Drug Administration under the Federal Food, Drug, and Cosmetic Act.[25]"

IFUS Point 5f(1d-1 Ref 30): https://en.wikipedia.org/wiki/Aspergillus_niger

IFUS Point 5f(1d-1 Ref 32): https://en.wikipedia.org/wiki/Beano_(dietary_supplement)

IFUS Point 5f(1d-1 Ref 24): https://www.gbif.org/species/144094364

IFUS Point 5f(1d-1 Ref 25): Diversity, Pathogenicity and Toxicology of A. niger: An Important Spoilage Fungi, Ajay Kumar Gautam, Research Journal of Microbiology, 2011, Volume: 6, Issue: 3, Page No.: 270-280

IFUS Point 5f(1d): "Fungal hydrolysis of ellagitannins produces hexahydroxydiphenic acid, which is considered an intermediate molecule in ellagic acid release. Ellagic acid has important and desirable beneficial health properties. The aim of this work was to identify the effect of different sources of ellagitannins on the efficiency of ellagic acid release by Aspergillus niger. Three strains of A. niger (GH1, PSH and HT4) were assessed for ellagic acid release from different polyphenol sources: cranberry, creosote bush, and pomegranate used as substrate." Effect of different polyphenol sources on the efficiency of ellagic acid release by Aspergillus niger," L. Sepúlveda, et.al., Rev Argent Microbiol. 2016 Jan-Mar;48(1):71-7. doi: 10.1016/j.ram.2015.08.008. Epub 2016 Feb 23.

IFUS Point 5f(1e): "Ellagitannase is a novel enzyme responsible for biodegradation of ellagitannins and ellagic acid production. Ellagic acid is a bioactive compound with great potential in food, pharmaceutical and cosmetic industries. This work describes the ellagitannase enzyme production from partial purified ellagitannins as inducers by Aspergillus niger GH1 grown on solid-state fermentation," (Potential use of different agroindustrial by-products as supports for fungal ellagitannase production under solid-state fermentation, Juan Buenrostro-Figueroa, et.al., Food and Bioproducts Processing, Volume 92, Issue 4, October 2014, Pages 376-382)

IFUS Point 5f(1f): In a study performed by Wenjie Zhang, et.al. ("Effects of dietary addition of ellagic acid on rumen metabolism, nutrient apparent digestibility, and growth performance in Kazakh sheep," Front Vet Sci. 2024 Feb 6;11:1334026. doi: 10.3389/fvets.2024.1334026), one finds that: "The EA group exhibited significantly higher dry matter intake ($p < 0.05$) and increased the digestibility of neutral detergent fiber and ether extract when compared with the CON group ($p < 0.05$). Additionally, the plasma activities of total antioxidant capacity (T-AOC), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) were significantly higher, while malondialdehyde (MDA) concentration was significantly lower in the EA group compared to the CON group ($p < 0.05$). In conclusion, dietary supplementation with 30 mg/kg BW EA in 5-month-old Kazakh sheep increased the dry matter intakQ16e, apparent digestibility of neutral detergent fiber, and ether extract, as well as the contents of acetic acid and propionic acid in rumen fluid. Moreover, EA supplementation regulated the ruminal microbiota, enhanced antioxidant capacity, and improved daily weight gain."

IFUS Point 5f(1g-1): Sheep are similar to bovines as "Sheep are ruminants, meaning they have four-chambered stomachs. Their unique digestive system consists of the rumen, reticulum, omasum, and abomasum. The main function of a sheep's ruminant digestive system is to allow better absorption of energy from food." The Sheep Stomach System Explained, Alexandra, Sheep Biology, August 23, 2022

IFUS Point 5f(1g): One can entertain that a reasonable extrapolation would suggest that the ellagic acid produced from microbial activity in Sugarcane Bagasse balances the gallic acid from SGP+™ to enable enhanced digestion and subsequent absorption, while suppressing methane, especially if the *in vitro* degradation and depolymerization of lignin continues *in vivo* in the Upper GI of the bovine. (See IFUS Point 5g(1))

IFUS Point 5f(2): In the early stages of SGP+™, industrial grade methane detectors used in chemical plants and oil refineries were used to sample for methane produced from newly acquired F-1 Heifers being introduced to SGP+™. These highly sensitive meters (used for "HOT WORK PERMITS" in chemical plants and refineries) could not detect CH4 when the cows were eliminating waste, or from the fresh manure on the ground, or from their mouths.

IFUS Point 5f(2-1): These CH4 tests were conducted every few days for 6-months on a herd of F-1 Brangus Heifers in an open pasture. The herd was introduced to an initial ration mix of 15% SGP+™ with pelletized feed and free-range grass. The SGP™ increased over time to 75% SGP+™ as part of total ration. At that point the Manure Pats transformed from Score 1 to Score 4/5 Pats. The ration was adjusted downward to 65% SGP+™ of total ration mix. Score 3 Pats were achieved. Where CH4 was initially detected from the heifers, once Score 3 pats were achieved through adjustments in ration mix, the presence of CH4 became undetectable. (NOTE: As an aside, herd feeding costs decreased from $1.41/lb/head/day to $0.41/lb/head/day with Herd Scoring improving as the heifers moved into estrous and then pregnancy.)

IFUS Point 5f(2-2): The results reported are believed to reflect lignin suppression of methane production: "Methane production through anaerobic digestion: Participation and digestion characteristics of cellulose, hemicellulose and lignin," Wanwu Li, Habiba Khalid , Zhe Zhu, Ruihong Zhang, Guangqing Liu, Chang Chen, Eva Thorin, "Applied Energy," Volume 226, 15 September 2018, Pages 1219-1228, https://doi.org/10.1016/j.apenergy.2018.05.055.

IFUS Point 5f(2-2a): This study also indicates that: "Anaerobic digestion characteristics of lignocellulosic components are described." "Lignin caused more severe inhibition on methane yield of cellulose than hemicellulose."

IFUS Point 5f(2-2b): Furthermore, additional studies determined the biochemical pathways whereby lignin is degraded and depolymerized by White Rot Fungi resulted in water production both *in vitro* and *in vivo*: “Lignin degradation: microorganisms, enzymes involved, genomes analysis and evolution,” Janusz G, et.al., FEMS Microbiology Reviews, 01 Nov 2017, 41(6):941-962, https://doi.org/10.1093/femsre/fux049 PMID: 29088355 PMCID: PMC5812493.

IFUS Point 5f(2-2b.1): Figures 1, 2, 3, and 4 progressively illustrate lignin degradation and depolymerization pathways by microbes contained in Sugarcane Bagasse, to include how methane is prevented from being produced in these particular pathways where lignin is involved in bovine digestion.

Fig.1

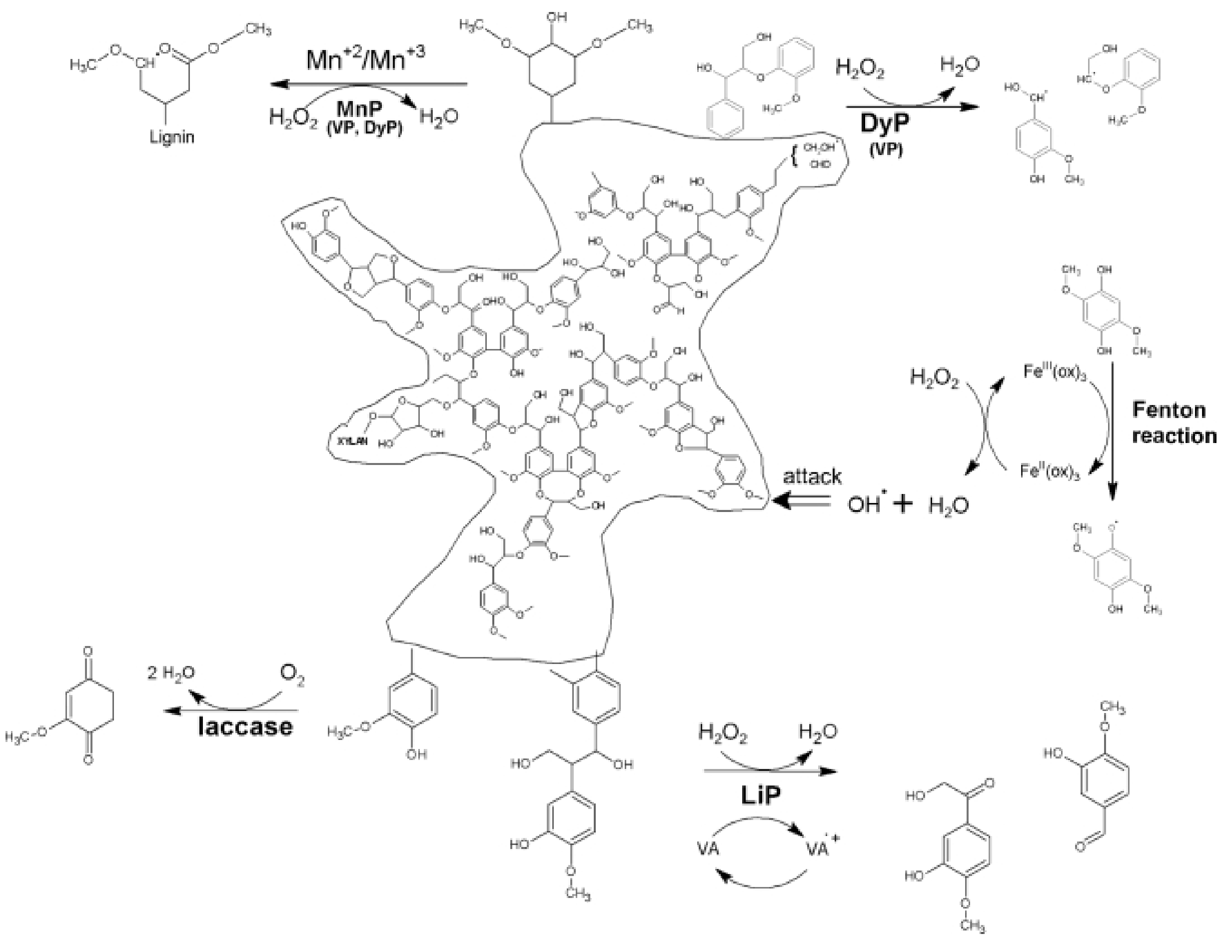
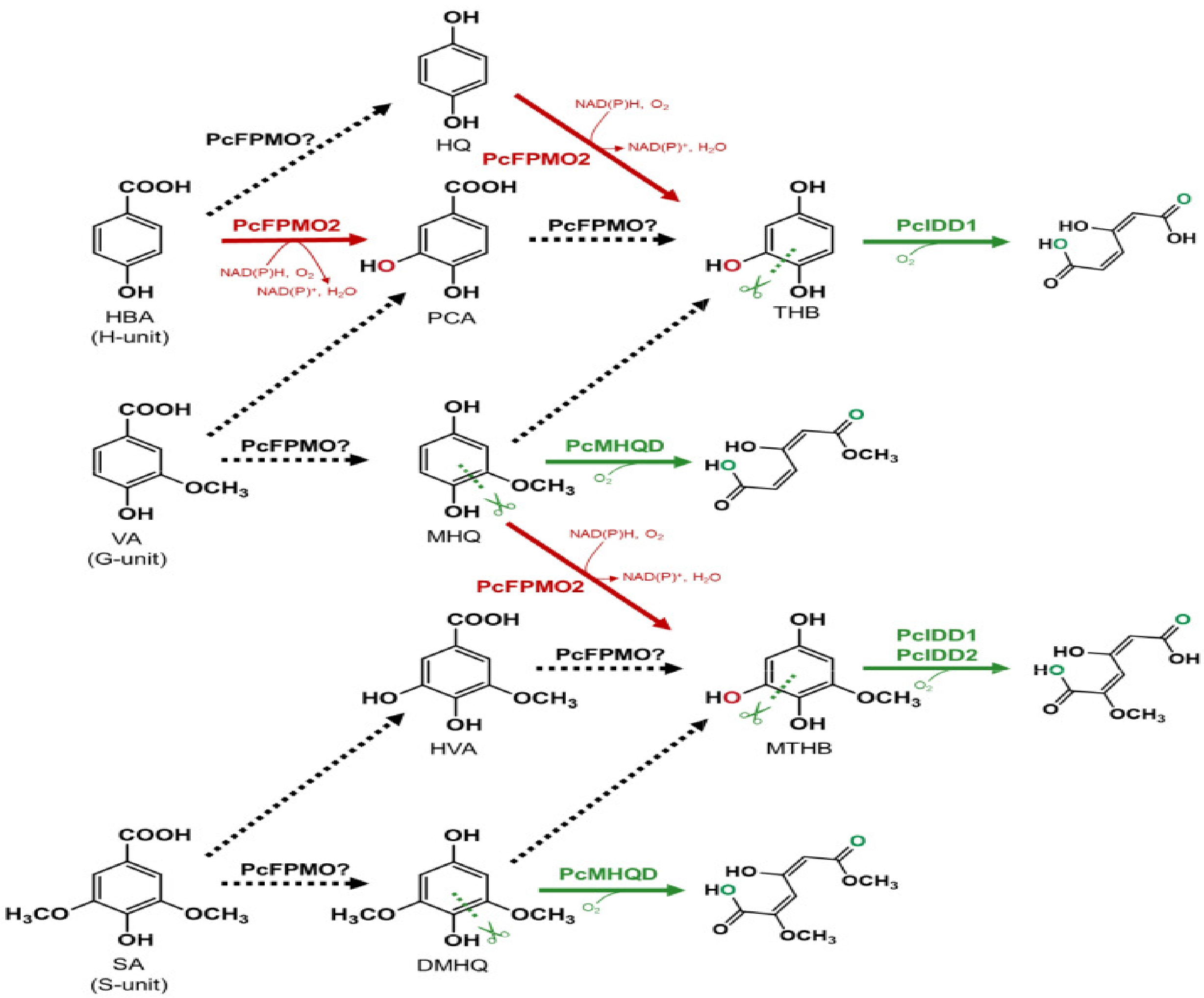
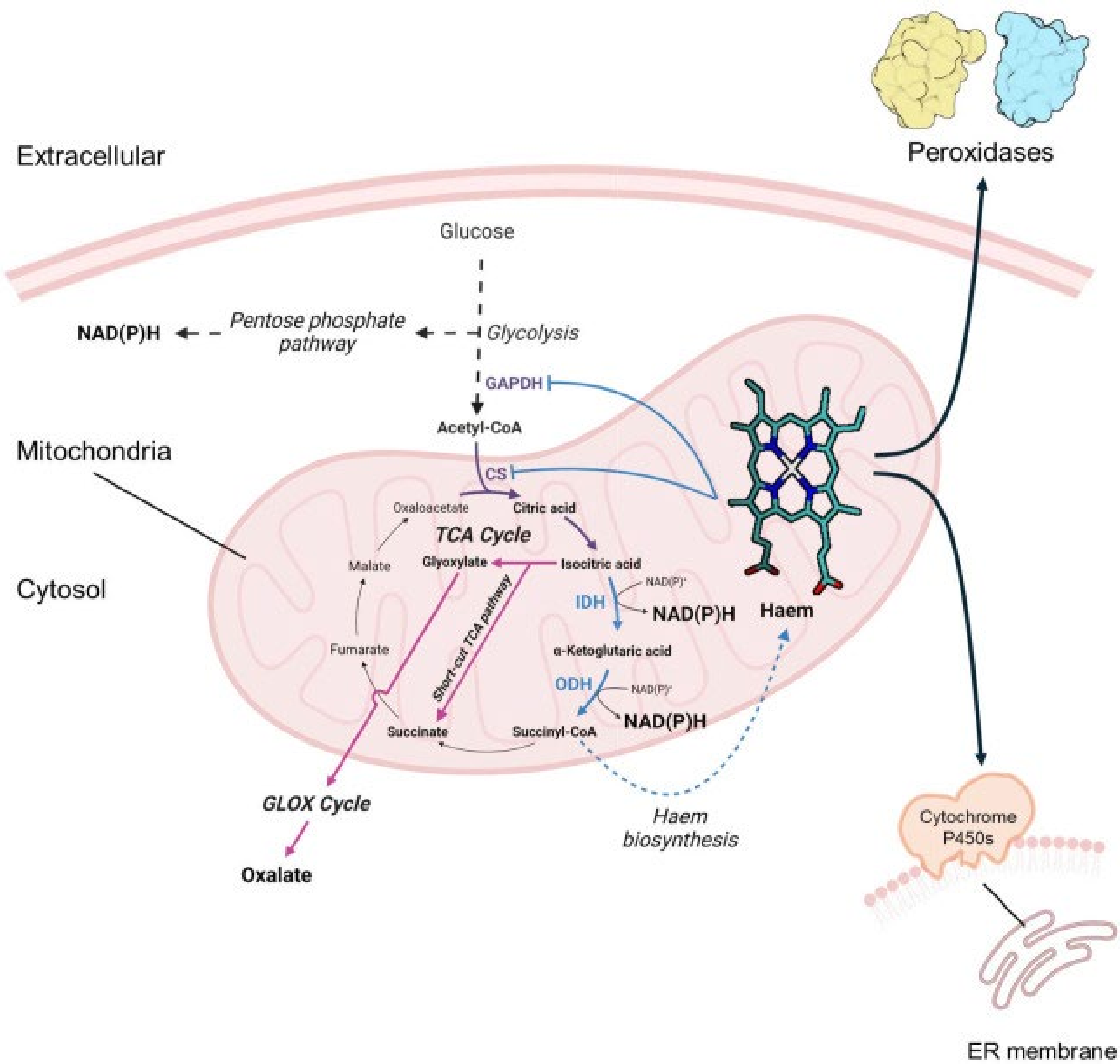


Fig. 2.



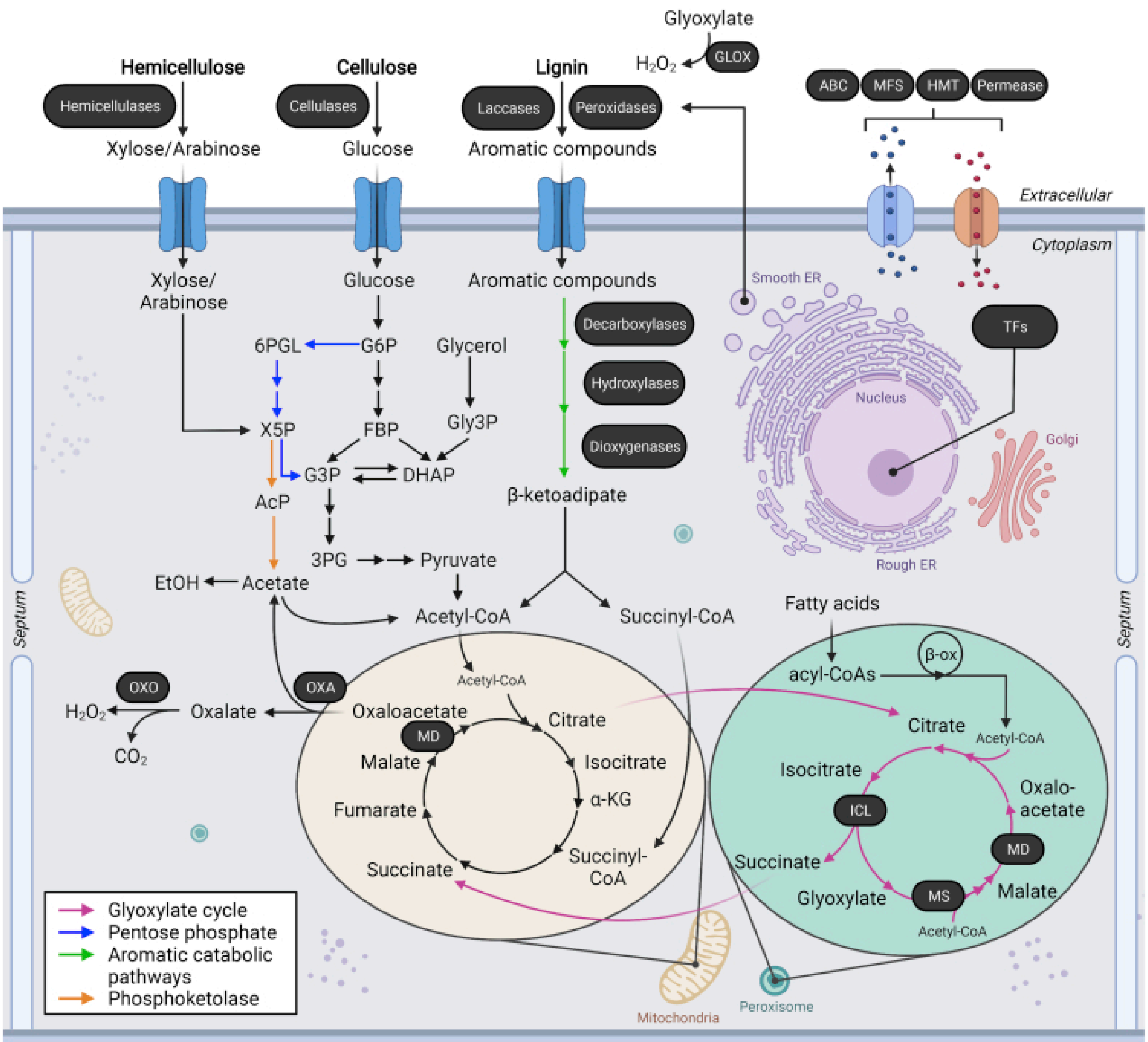
Metabolic pathways of HBA, VA, and SA in white-rot fungus *P. chrysosporium*. Dotted arrows indicate the estimated reactions, and solid arrows indicate the reactions by identified enzymes including flavoprotein monooxygenase 2 (PcFPMO2), MHQ dioxygenases (PcMHQD), intradiol dioxygenase 1 (PcIDD1), and intradiol dioxygenase 2 (PcIDD2) (Appl Microbiol Biotechnol. 2024 Dec 11;108(1):532. doi: 10.1007/s00253-024-13371-4)

Fig. 3.



Central metabolic regulation of white-rot fungi in lignin degradation. During degradation of lignin and lignin-derived aromatics, production of isocitrate dehydrogenase (IDH) and α-ketoglutarate dehydrogenase (ODH) significantly increases, resulting in a shift in metabolic flux from a short-cut tricarboxylic acid (TCA)/glyoxylate bicycle system (magenta) to the classical TCA cycle (cyan), which activates the haem biosynthetic pathway by providing succinyl-CoA for effective production of haem enzymes. In contrast, excessive haem production directly inhibits citrate synthase (PcCS) and glyceraldehyde 3-phosphate dehydrogenase (PcGAPDH), thereby regulating haem synthesis, ATP synthesis, flux of the TCA cycle, and NADPH production. Through this metabolic regulation, effective production of haem enzymes, including lignin and manganese peroxidases and cytochrome P450s, takes place. This figure was generated using BioRender (https://biorender.com/) (Appl Microbiol Biotechnol. 2024 Dec 11;108(1):532. doi: 10.1007/s00253-024-13371-4)

Fig. 4



Summary of proposed intracellular catabolic pathways during lignocellulose degradation in WRF

Extracellular cellulose-, hemicellulose-, and lignin-degrading enzymes break down biopolymers from lignocellulose into low molecular weight products (e.g., glucose, xylose, aromatic compounds) which are subsequently funneled to the tricarboxylic acid and glyoxylate shunt pathways. Solid arrows represent enzymatic reactions and faded arrows represent transport in or out of organelles. Key proteins and enzymes highlighted in this review are shown in dark gray ovals. Metabolic compound abbreviations: 3PG, 3-phosphoglycerate; 6PGL, 6-phosphogluconolactone; AcP, acetyl phosphate; DHAP, dihydroxyacetone phosphate; EtOH, ethanol; FBP, fructose 1,6-bisphosphate; G3P, glyceraldehyde 3-phosphate; G6P, glucose-6-phosphate; GLOX, glyoxal oxidase; X5P, xylulose-5-phosphate; Gly3P, glycerol-3-phosphate. Enzyme and protein abbreviations: ABC, ATP-binding cassette efflux transporters; HMT, heavy metal translocation protein; ICL, isocitrate lyase; MD, malate dehydrogenase; MFS, major facilitator superfamily; MS, malate synthase; OXA, oxaloacetase; OXO, oxalate oxidase; TFs, transcription factors. "Systems biology-guided understanding of white-rot fungi for biotechnological applications: A review," Teeratas Kijpornyongpan, et.al., iScience. 2022 Jun 18;25(7):104640. doi: 10.1016/j.isci.2022.104640.

IFUS Point 5f(3):: As the SGP+™ is stacked, water continues to form on the wrapping and leaks from the highly compressed 2000-lb bales. Furthermore, Joe Wilcox (a rancher on the OK/KS-border) has actually measured a decrease in water intake from his herds, while noting that the heat tolerance of the herd has improved as well as decreased mortality rates of adults and calves.

IFUS Point5f(4): Furthermore, "A foundational study conducted by scientists at the National Renewable Energy Laboratory (NREL) shows for the first time that white-rot fungi are able to use carbon captured from lignin as a carbon source." "What we have demonstrated here is that white-rot fungi can actually utilize lignin-derived aromatic compounds as a carbon source, which means they can eat them and utilize them to grow," Salvachúa said. "That is another strategy for carbon sequestration in nature and has not been reported before." "Intracellular pathways for lignin catabolism in white-rot fungi," Carlos del Cerro, Erika Erickson, Tao Dong, Davinia Salvachúa, et.al., Proceedings of the National Academy of Sciences, February 23, 2021, 118 (9) e2017381118 https://doi.org/10.1073/pnas.2017381118 and https://orcid.org/0000-0003-0799-061

IFUS Point5f(5): (Note: From a polymeric standpoint, "Degradation" and "Depolymerization" are two VERY different physical and chemical processes and should NOT be interchanged or confused. In this case the scientists are finding these results from the degradation of lignin that aides the depolymerization of lignin, The latter produces the majority of the actual chemistry impacting the biochemistry of bovine ration management.)

IFUS Point 5f(6): The H2 produced reacts with O2 with the interaction of Mn and Fe peroxidases to create the water. Numerous studies show the presence of both Mn-Peroxidase and Fe-Peroxidase in Sugarcane Bagasse: "MnP is a heme peroxidase produced by white rot basidiomycetes fungi land expresses the oxidation of phenolic compounds in the presence of Mn(II) and H2O2. In the MnP catalyzing oxidation, chelate complexes of Mn(III) with organic acid such as malonate, lactate, or tartarate oxldize phenolic compounds, including lignin." Wariishi, H., Valli, K., Gold, M.H., 1992. Manganese (II) oxidation by manganese peroxidase from basidiomycete Phanerochaete chrysosporium, kinetic mechanism and role of chelators. J. Biol. Chem. 267, 23688-23695.

IFUS Point 5f(7): "Hydrolysis, acidogenesis, and methanogenesis are the main components of AD. Hydrolysis is generally considered to be the limiting step in the AD of lignocellulosic biomass. The presence of lignin prevents the AD of lignocellulose to produce methane, primarily by restricting the hydrolysis process.12,49 It was found that when the concentration of dissolved lignin was 5 g/L, the hydrolysis, acetogenesis, and methanogenesis activities decreased by 35%, 10%, and 15%, respectively, compared with the control." "Deciphering the Impact of Lignin on Anaerobic Digestion: Focus on Inhibition Mechanisms and Methods for Alleviating Inhibition," Ziyan Sun, et.al., ACS Omega. 2024 Oct 22;9(44):44033–44041. doi: 10.1021/acsomega.4c04375.

IFUS Point 5f(8): "Increasing the proportion of non-H2 producing fibrolytic microorganisms might decrease methane production without affecting forage degradability. Alternative pathways that use electron acceptors other than CO2 to oxidise H2 also exist in the rumen. Bacteria with this type of metabolism normally occupy a distinct ecological niche and are not dominant members of the microbiota; however, their numbers can increase if the right potential electron acceptor is present in the diet. "Microbial ecosystem and methanogenesis in ruminants," D.P. Morgavi, E. Forano, C. Martin, C.J. Newbold, Animal, Volume 4, Issue 7, 2010, Pages 1024-1036, https://doi.org/10.1017/S1751731110000546

IFUS Point5f(9): Both the mastic and carob used in the SGP+™ formulation contain gallic acid and small quantities of beneficial tannins for bovine health. "Use of gallic acid and hydrolyzable tannins to reduce methane emission and nitrogen excretion in beef cattle fed a diet containing alfalfa silage," Isaac A Aboagye, et.al., J Anim Sci. 2019 Apr 29;97(5):2230-2244. doi: 10.1093/jas/skz101.

IFUS Point 5f(10): The aforementioned is not even a fraction of the studies IFUS has reviewed, but a mere sampling of research that supports the claims made by ranchers and dairymen applying SGP+™ as part of a Ration Management Technology.

IFUS Point 6: **SUMMARY:** While valuable in providing some level of baseline measurement, Forage Analysis, Manure Analysis, and other such establish methods were not and are not designed to explain lignin depolymerization in the gut of the bovine. However, Manure Scoring reconciled to observable Herd Performance is telling a story somewhat different to what this Manure Analysis is suggesting. It DOES NOT suggest that the analysis is incorrect. It DOES suggest that these analyses cannot completely explain the efficacy of SGP+™.

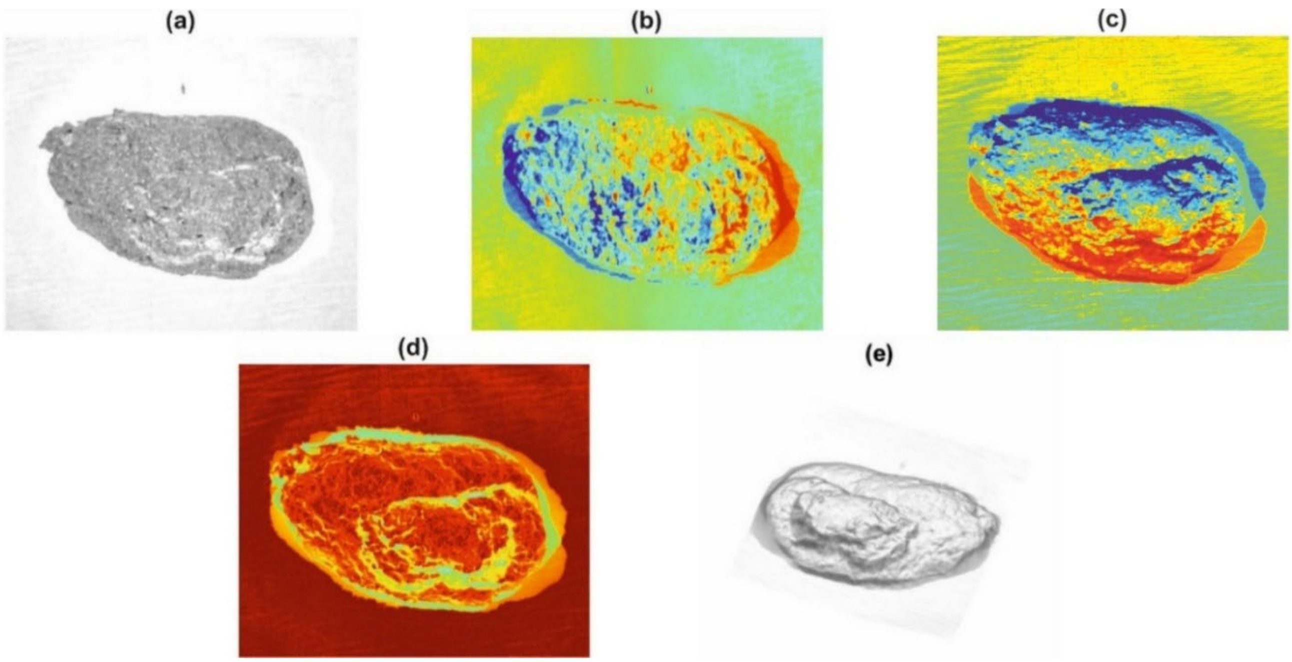
IFUS Point 6b: L.S.U. / Cumberland Analytical Services Forage to Manure Analyses: A series of Forage analyses performed by L.S.U. and Cumberland showed Forage Report showed Sugarcane Bagasse used in the formulation of SGP+™ to contain roughly 28% lignin. However, the lignin concentration decreased in an 85% SGP+™/15% Cracked Corn to 19.9%. Furthermore, in Manure Analysis performed on Score 3 Manure Pats resulting for the 85/15 mix, showed a further decrease in linin content to 7-9%.

IFUS Point 6b(1): This is indicative of the effect of White Rot Fungi and other beneficial microbes degrading and depolymerizing the lignin within the SGP+™ formulation. However, Forage /Manure/and similar analyses CANNOT determine what is occurring with lignin depolymerization occurs *in vivo.*

IFUS Point 6b(2): Manure Scoring reconciled to Herd Performance is telling a story as to the efficacy SGP+™.

IFUS Point 6b(3): Additionally, Manure Scoring is further supported by scientific advancements like, "A computer vision approach to improving cattle digestive health by the monitoring of faecal samples," Gary A. Atkinson, et.al., Scientific Reports volume 10, Article number: 17557 (2020) (See Figures 2 & 11)

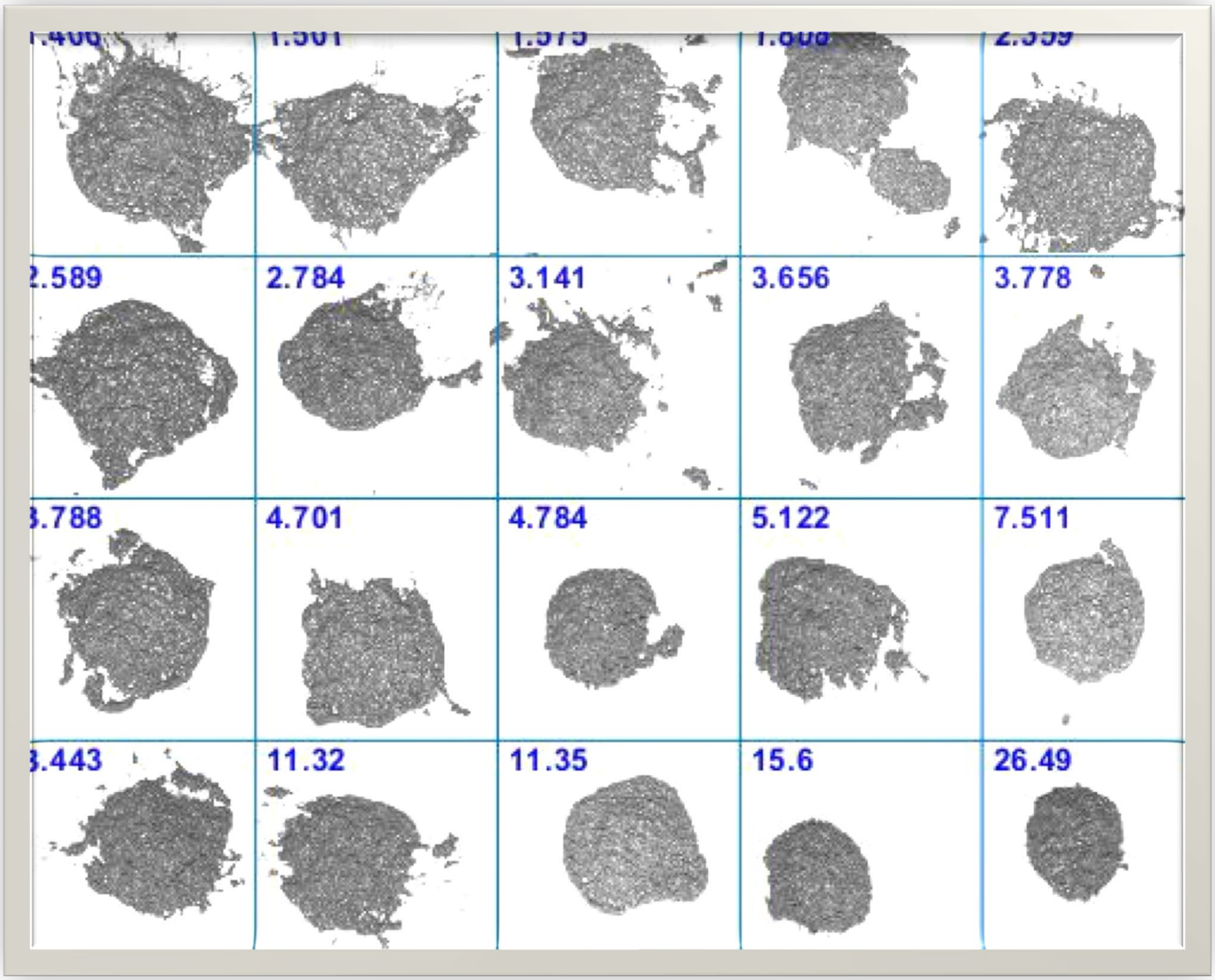
Figure 2 From: A computer vision approach to improving cattle digestive health by the monitoring of faecal samples



Sample data captured from the PS rig shown in Fig. 1. (**a**) Albedo. (**b**–**d**) x, y and z components of surface normals respectively. (**e**) Depth. NIR illumination was used for this figure.

Figure 11

From: A computer vision approach to improving cattle digestive health by the monitoring of faecal samples



Images of samples according to calculated total score (score values above-left of each sample).

IFUS Point 7 **CONCLUSIONS**:

IFUS Conclusion 1: IFUS NEVER intended SGP+™ to create the Herd Performance being reported to us by ranchers and dairymen. Hence, the science we have been doing is an attempt to understand what is happening after the fact. Furthermore, where IFUS has analyzed Sugarcane Bagasse, Sugarcane Bagasse with only Carob, and SGP+™, improvements are in fact indicated by Forage Analysis, I.V.T.D., Manure Analysis, and the like. However, none of these standard tests explain or support the Herd Performance being reported to us.

IFUS Conclusion 2: IFUS applies Mastic Oil, Carob, Water, and Ionic Minerals to economically depolymerize Sugarcane Bagasse into a useful ration, that when blended with cracked corn, DG, Sugarcane Tops, and the like, allows ranchers and dairymen to lower their respective cattle feed costs from up to \$3.50/lb/head/day to as little as \$0.41/lb/head/day.

IFUS Conclusion 3: The degrading and depolymerization of lignin begins in formulation as evidenced by:

a) Shifts in steaming bagasse (135-145°F), transforming the material (SGP+™) to lowered temperatures (92-94°F), with continued cooling (84-86°F) within 2000-lb bales while sitting in the warehouse. Lignin depolymerization is shown to be endothermic.

b) Like hay, when bagasse bales are stacked one atop the other, the structural integrity of the bale remains intact for ages. This is due to the lignin within the bales. However, when SGP+™ bales are stacked one atop the other, within 90-days the bales collapse. A notable difference in the fiber structure becomes visible as well as the feel to the fiber. Examination under microscopy also illustrates a shift in fiber morphology.

c) H20 produced from degraded/depolymerized lignin shown to (1) suppress methanogenesis and (2) in a separate acid/base reaction individually and possibly collectively result in improved hydration, and quicker satiation of cattle (chewing cuds 25% quicker in morning and evening feedings), while reducing Greenhouse Gases though *in vivo* reduction of methanogenesis, accordingly.

d) Testing performed by the L.S.U. Southeast Research Station Forage Quality

Lab demonstrated a marked I.V.T.D. improvement of SGP+™ as compared to Sugarcane bagasse (47.5% vs 32.0%, respectively). Where by this analysis alone SGP+ should NOT produce the Herd Performance claimed by ranchers and dairymen, per Dr. Mike McCormick, it does beg the question as to what caused this significant improvement in I.V.T.D.

e) Forage / Manure Analysis Comparison suggests SGP+™ that CP/TDN is being digested, absorbed with ample energy for Bovine Heard Performance: A series of Forage analyses performed by L.S.U. Southeast Research Station Forage Quality Lab and Cumberland Valley Analytical Services showed Sugarcane Bagasse used in the formulation of SGP+™ to contain roughly 28% lignin. However, the lignin concentration decreased in an 85% SGP+™/15% Cracked Corn ration mix to 19.9%. Furthermore, in Manure Analysis performed on Score 3 Manure Pats resulting for the 85/15 mix, showed a further decrease in lignin content to 7-9%. Cumberland also noted undigested cracker corn in the original manure sample.

IFUS Conclusion 4: Degraded / Depolymerized lignin shown to produce water, suppress methane, minimize acidosis, provide ample crude protein as well as proteinogenic amino acids, and more than ample TDN for Herd Performance, Milk Production, and more.

IFUS Conclusion 5: Herd Manure Scoring, per Dr. Robert Wells, formerly of the Nobel Research Institute and now Professor of the Practice and Paul C. Genho Endowed Chair in Ranch Management, King Ranch Institute (https://krirm.tamuk.edu/robert-wells-ph-d-pas/), reconciled to Herd Performance has shown that Score 3 Manure Pat contain: “12-15% CP; 62-70% TDN of diet.”

IFUS Conclusion 5-1: Manure Scoring is further supported by scientific advancements like, “A computer vision approach to improving cattle digestive health by the monitoring of faecal samples,” Gary A. Atkinson, et.al., Scientific Reports volume 10, Article number: 17557 (2020)

IFUS Conclusion 6: The symbiotic and synergistic formulation of Mastic, Ionic Minerals, Water, Carob, and Sugarcane Bagasse, in concert with a healthy Fungal and Bacterial Biome both *in vitro* and *in vivo* results in:

- a ration more easily digested requiring less chemical secretion from the bovine (degraded/depolymerized lignin with carbohydrates, fats/lipids, and minimal protein).
- enhanced absorption (ionic minerals).
- improved hydration (mineralized water).
- re-established natural gut flora (the latter providing 80% of the protein

required by the bovine).
- Ingredients formulated to produce natural anti-inflammatory and anti-biotic / probiotic shown to exist in each of the ingredients added into the formulation.

IFUS Conclusion 7: Flies and fly larvae are not found on the manure pats resulting from ration mix with 80+% SGP+™. Screw Worm Flies may be repelled by tannic substances produced from the digestion of SGP+™, which contains Mastic, Ionic Minerals, Water, Carob, and Sugarcane Bagasse.

IFUS Conclusion 8: Estrogenic precursors are produced from degraded/depolymerized lignin from Sugarcane Bagasse, and are found in Mastic and Carob while being supported by ionic minerals.

IFUS Conclusion 9: Present-day analysis methods, where proven and a solid starting point, are not adequate to demonstrate the efficacy of SGP+™ as part of an overall Ration Management Strategy. However, it would seem that Manure Scoring reconciled to Herd Performance along with Forage Analysis followed through to Manure Scoring may be serving ranchers and dairymen in producing improved results for their respective herds.

IFUS Conclusion 10: Where incredible science and discipline in its application has served the beef and dairy industries well, new challenges will require a C-Change in thinking in ration management, and NOT another quick-fix additive or GMO on the feed or the cow itself. Nature provides the clues, the data, and the results to direct us to solutions if we open our minds and study/implement these solutions.

Lastly, please note that the science offered in this White Paper does not even scratch the surface of the myriad of studies performed on Sugarcane Bagasse and delignification. However, these studies when taken into context and overall consideration do present per IFUS' perspective substantive and plausible explanations as to the efficacy of SGP+™ as an integral part of improved Bovine Ration Management.

IFUS Point #8: Next Steps

Study 1:
Step 1a: Forage Analyze

- Sugarcane Bagasse
- SGP+™
- SGP+™/Added Deer Run Ranch Ration

Step 1b: Manure Score and Analyze

- Manure Score Pats on Herd fed SGP+™/Added Deer Run Ranch Ration
- Manure Analyze Deer Run Ranch Manure Pats on Herd fed SGP+™/Added Deer Run Ranch Ration

Step 1c: Photograph

- Coats / Finish of Heifers and Calves
- Milk Bags of Heifers
- Flies (or lack thereof) on Heifers/Calves as well as Manure Pats
- Fly larvae in Manure Pats

Step 1d: Video

- Stirring Manure Pats for fly larvae
- Calf behavior and wellness

Step 1e: Daily Documentation

- Grazing time in Morning and Evening before chewing cuds
- SGP+™/Other Ration Daily ratios and amount fed for total herd (gather a rough count of heifers eating the ration each day).

Step 1f: Other relatively easy analysis and documentation recommended by A&M; e.g., BUN, MUN, etc.

Study 2: Steps 1a-1e repeated on a blind trial of 30 Non-lactating heifers (or some equivalent standard) with 10 Heifers per Herd for 6-9 months.
Herd 1: Fed Normal Ration
Herd 2: Fed Sugarcane Bagasse introduced at 15% of ration mix and increase by an additional 15% until an 80%/20% ration mix achieved.
Herd 3: Fed SGP+™ introduced at 15% of ration mix and increase by an additional 15% until an 80%/20% ration mix achieved.

Study 3: Steps 1a-1e repeated on a blind trial of 50 Non-lactating heifers (or some equivalent standard) with 10 Heifers per Herd for 6-9months.
Herd 1: Fed Normal Ration
Herd 2: Fed Sugarcane Bagasse introduced at 15% of ration mix and increase by an additional 15% until an 80%/20% ration mix achieved.
Herd 3: Fed SGP+™ introduced at 15% of ration mix and increase by an additional 15% until an 80%/20% ration mix achieved.
Herd 4: Fed Sugarcane Bagasse with only Carob added and introduced at 15% of ration mix and increase by an additional 15% until an 80%/20% ration mix achieved.

Herd 5: Fed Sugarcane Bagasse with only Nutri-Mastic™ added and introduced at 15% of ration mix and increase by an additional 15% until an 80%/20% ration mix achieved.

Study 4: Add on to Study 3 & 4 with Carcass Analysis, etc.

IFUS Point #9: IFUS Embedded Comments in Deer Run Ranch Manure Report. Again, please note that these are offered in the spirt of the search for the truth and in NO WAY a criticism of the A&M Team or their work!

IFU

Hereyou go Dalette.

FYI I used my software to help create the report.

IFUS Note: Comments below are NOT criticisms of the work performed in this report, but comments for consideration as to what SGP+™ is and how it might impact Bovine Ration Management and Herd Performance.

Feed Performance and Intake

This section looks at how the **feed is performing** in terms of cow weight, protein intake, and overall consumption of feed (dry matter). The good news: **all cows maintained their weight** on the bagasse/DDG diet, and their protein needs were met. Here are the key points:

- **Cow Weight and Body Condition:** All sampled cows weighed around **1,250-1,300 lbs** and had a **Body Condition Score ~6** (on a 1-9 scale). A BCS of 6 means they were in **good flesh** - neither too thin nor overly fat, which is ideal for cows nursing calves. Maintaining this condition indicates the feed is keeping them healthy.
    - **Considering that Deer Run Ranch's Business Model is to purchase**

low scoing cattle, the transformation into Score 6 is notable. Also, Dr. Pat Bagley of SURAEC visited the ranch in Mid-Summer of 2024 and scored the Herd as improved from Score 1-2 to Score 4-5.

- **Weight Change:** The goal was to keep weight stable (no gain or loss). The lab's model predicted essentially O **lbs/day change** for all three samples (one was -0.02 lbs/day, effectively zero). In plain terms, **cows are holding their weight** on this diet - they're not losing weight (a sign of insufficient feed) and not gaining excess weight either [25t I
    - **Considering that the heifers have achieved their adult weight, then no further weight gain would be desired, correct?**
- **Dry Matter Intake:** Each cow ate roughly **29-30 lbs of dry matter per day** in total. For perspective, that's a normal, healthy amount for a cow of this size. About **5.8 lbs** of that was distillers' grains (the high-protein part of the ration), and the rest (~23-24 lbs) was the bagasse-based forage. This means the cows are willing to eat the bagasse mix in significant amounts, which is important since bagasse is a very fibrous feed.
    - **Bagasse is only one ingredient in SGP+™. Hence, where Bagasse is a ND40 Fiber with an I.V.T.D. of 32.0s, SGP+™ has an I.V.T.D. of about 47.5. Also, the addition of DG to the ration mix is very recent. Until now the SGP+™ to Cracker Ccrn mix has been reported nby Deer Run ranch to be roughly 80/20, respectively. Yet, Deer Run ranch has reported a decrease in grazing time.**
- **Distillers Grains Consumption:** All samples show the cows were fed about **5.8 lbs per day of distillers grains (DDG)** per head (as-fed). This supplement is the primary protein source in the TMR. The consistent intake across samples suggests a stable feeding program – every cow got her share of DDG to balance the low-protein bagasse.
    - Based on the mis-understanding as to what SGP+™ is, this conclusion is appropriate. However, with a better understanding of SGP+™ per the information provided in the IFUS Comment section, then a reconsideration of this comment may be warranted.
- **Protein Intake vs Requirement:** Despite the low-quality fiber in bagasse, the DDG supplement ensured the cows' **protein needs were met or slightly exceeded.** For example, one sample (ID 168703) had a **crude protein intake of ~2.78 lbs/day** against a requirement of **2.54 lbs/day,** leaving a small **surplus of ~0.24 lbs** of protein. In all three samples, protein intake was at or above what the cows need for maintenance and early lactation. This means the 80/20 diet is giving enough protein, largely thanks to the distillers grains.
    - Again, SGP+™ is NOT simply bagasse as bagasse in only one component of SGP+™. Again, in consideration ot the IFUS Commentary above, this comment may prove necessary. Furthermore, the addition of DG to the ration mix is a recent development. Until about a month or so ago the ratio mix had been modified to 90% or SGP+™ to 10% Cracked Corn (or so reported by Deer Run Ranch).
- **Energy (Digestible Feed) Intake:** All cows' performance was **"limited by energy"** rather than protein. Bagasse is bulky and not very energy-dense. The **Digestible Organic Matter (DOM)** of the diet was around **50%,** which translates to a moderate energy level (roughly equivalent to a Total Digestible Nutrient value in the mid-50s%). In practical terms, the cows are filling up on the TMR but reaching

the limit of energy intake – they can't eat much more to get extra energy because bagasse fiber is so filling. That's why the model showed **no weight gain** and why **milk output didn't hit maximum potential** (more on that below). Simply put, **energy from the feed was just enough, but not abundant.**

- Again, SGP+™ is NOT simply bagasse as bagasse in only one component of SGP+™. Again, in consideration ot the IFUS Commentary above, this comment may require reconsideration.

Manure Characteristics (Nutrients & Output)

Now let's talk about **what's in the manure** - particularly nitrogen (N) and phosphorus (P) - and how much manure is coming out daily. These manure results tell us how well the cows are using the feed and give hints about environmental factors like soil nutrient return, flies, and even methane.

- **Total Manure Output:** Each cow produced roughly **12-13 lbs of manure (dry matter) per day.** That's the dried equivalent of the manure; in fresh weight it would be much higher because manure contains a lot of water. (The water content of a Score 3 pat is significantly lower than a Score 1-3.)With about 29-30 lbs of feed in, about 12 lbs out as dry manure indicates roughly **60% of the feed dry matter was digested,** and **40% came out.** This aligns with the **~50% DOM** figure - about half the feed was digestible and the other half remained in the dung. So, the cows are extracting what they can from this fibrous diet and dropping the rest in the pasture. (Again, without actually measuring what specifically is in the manure, and based on what is being suggested by Manure Score 3 Pats, Herd Performance as reported by Deer Run Ranch, and the science discussed above, this conclusion may warrant reconsideration.
- **Fecal Nitrogen (N):** Nitrogen in manure is basically protein that wasn't used by the cow. Two of the samples (168704 and 168705) had manure nitrogen around **1.25-1.29%** of the dry manure. The other sample (168703) was higher, about **2.3% N.** To put that in daily terms, cow 168703 dumped about **0.25 lbs of N per day** in manure, while the others were closer to **0.13 lbs N per day.** The higher Nin sample 168703's manure could mean that cow didn't use all the protein she ate (perhaps she ate a bit more protein or produced a bit less milk, leaving extra N to excrete). The others have lower fecal N, which often means **more of the feed protein was absorbed and used** (good for the cow, less N wasted). (This assumes that the N-metabolism is as once expected and not shifted by the degradation and depolymerization of lignin and the added protein content shown to be produced by this process *in vitro* (prior to consumption) and *in vivo* (during and after consumption.)
- **Fecal Phosphorus (P):** Phosphorus is a key mineral for cattle and also a concern for runoff pollution. The manure P content varied: one sample (168704) was about **0.32% P,** while the other two were **0.21% and 0.27% P.** In practical terms, **0.3% or above is considered adequate dietary P** for a cow. So cow 168704's manure suggests she had enough phosphorus in her diet (and excreted the excess), whereas the others were on the borderline low side. This could be natural variation; all cows were likely fed the same, but small differences or how each cow's body used P can change the manure levels. Overall, there's **no glaring phosphorus deficiency** - but we see that not much Pis wasted either. Each cow excreted only around **0.02-0.03 lbs of P per day** in manure, which is actually quite modest. (Or this could also be an effect of enhanced digestion and absorption of the P. This is a point that IFUS must consider further.)

- **Manure Consistency and Fiber:** Although not a measured number in the report, the figures above imply the manure had a **high fiber content** (since roughly half the diet wasn't digested). You'd expect these manure piles to be fairly bulky and fibrous. Ranchers might notice **drier, fibrous patties** when cows eat something like bagasse. This can be a good thing for soil (adds organic matter) and might affect fly breeding (less soupy manure, possibly fewer flies - more on that next). (Analysis from Cumberland Valley Analytical Services shows a lower-than-expected fiber count when considering Sugarcane Bagasse to SGP+™ to manure. Also Score 3 Pats are NOT indicativ of high fiber content, nor are the lack of fly larvae in the Manure Pats.

Fly Control and Methane Clues

The report doesn't directly measure flies or methane, but we can **infer some effects** from the feed and manure data:

- **Fly Control:** Flies tend to thrive in **wet, nutrient-rich manure** (especially with lots of undigested protein that creates odors like ammonia). In these samples, manure nitrogen was not extremely high, and the manure is likely quite fibrous and a bit drier due to the bagasse. This could mean **less ammonia smell** and a manure texture that's **less ideal for fly larvae.** In plain language, the cow pies from this diet might crust over faster and be less stinky than those from cows on high-protein lush pasture. That's potentially **good news for fly control** - fewer flies bothering the herd and the ranch family. While the lab report doesn't say "fly count," the nutrient profile hints that this **bagasse diet could make manure a bit less attractive to flies** compared to higher-protein diets.
    - The science tells us that flies can detect manure within 2 seconds of excretion. This is believed to be due to the furfurals produced in the lower GI of Bovine digestion from undigested lignin and other undigested/unabsorbed nutrients that bacteria attempt to breakdown. Once attracted to the manure, the fly is said to place its proboscis into the manure to sample for nutrients required for its larvae to grow once hatched from eggs. Undigested lignin in the manure is converted by microbes into the nutrients required by the fly. If no nutrients are found, they fly is said to seek these nutrients elsewhere. Furthermore, when lignin is digested in the Upper GI of the Bovine, science is suggesting that flavonoids are produced, which are known to reply insects, to include flies.
    - Also, “In this context, the improvement of the diet of ruminant species with polyphenols and the influence of these compounds on animal performance, biohydrogenation processes, methanogenesis, and quality and quantity of milk have been extensively investigated through in vitro and in vivo studies.”
        - Marialuisa Formato, et.al., Polyphenols for Livestock Feed: Sustainable Perspectives for Animal Husbandry? Molecules. 2022 Nov 10;27(22):7752. doi: 10.3390/molecules27227752

- **Methane Reduction:** Cows produce methane during digestion, especially when fermenting high-fiber feeds. The data show a **moderate digestion rate (~50% DOM),** meaning a lot of fiber is fermented in the rumen. Usually, more fiber fermentation can lead to more methane per pound of feed. However, because bagasse is low-quality, the cows can only eat so much - they max out at ~30 lbs dry intake. So total methane might be limited by intake. There's no direct methane measurement, but one indicator is the **DOM/CP ratio.** All samples had quite high DOM/CP ratios (around 8 to 10, which is above the optimal 4-6 range for rumen efficiency). A high ratio like this suggests the microbes might be a bit starved for protein relative to energy, which can slow their growth. Sometimes that can lead to more methane per unit of feed (inefficient rumen fermentation). In simple terms, **the diet's fiber is fermenting, but not super efficiently.** If the goal was methane reduction, results are mixed: the cows aren't eating an excessive amount, but the fiber they do eat might ferment in a way that produces some methane. Without a direct measurement, we'd say **methane output is about what we'd expect for a high-fiber diet** - probably not significantly reduced, but the feed's intake limit naturally caps total methane somewhat.

- **Based on the science offered by IFUS above, this statement may need reconsideration.**

(Bottom line: the new bagasse feed isn't obviously causing a fly explosion or anything - manure might even be less fly-friendly. As for methane, the diet doesn't eliminate methane (since it's still a lot of fiber fermenting), but at least the cows can't overeat on it, which keeps total emissions in check.)

Cow Health and Milk Production Indicators

These tests give us a peek into the **cows' health and production,** beyond just weight:

- **Body Condition & Weight:** As mentioned, all cows were in good body condition (score 6) and maintained weight. No signs of weight loss means **the feed is meeting their maintenance needs,** even in early lactation when cows are under stress to produce milk.
- **Milk Production Potential vs Actual:** The lab report estimated each cow's **milk production potential** versus what the diet can actually support. For example, one

cow had the genetic or inherent potential to give about **18.6 lbs of milk per day,** but based on the energy and nutrients she was actually eating, the model predicted **she could actually produce about 14.4 lbs/day** on this diet. All samples showed a gap like this - the **"Actual" milk supported was roughly 75%-80% of the cow's potential.** This isn't surprising; bagasse is a low-energy roughage, so the cows are producing somewhat less milk than they might on a richer diet. However, ~14-15 lbs of milk a day is still a solid amount for a beef cow nursing a calf (that's roughly 1¾ gallons of milk). The calves should be healthy, though the cows aren't hitting their theoretical peak milk. If more energy (like grain or better forage) was provided, they might milk closer to potential. But the current output is **adequate for raising calves** without overly taxing the cows.

- A 15-day trial monitored by DVM's at the SUMUL Dairy in Surat, India on 2 Non-lactating Holstein-Friesian (HF) Crossbred Heifers (ages 4 and 3.5 years) at 940lbs and 748 lbs, respectively showed Manure Score improved after 2 weeks from Score 1 to Score 2.5. Where Milk fat percentage were the same, SNF (Solid-non-fat) percent increased by 0.6-0.8%. SNF typically ranges around 8%. A 0.6-0.8% increase = 7.5 – 10.0% increase in SNF. Hide shine improved after 10 days. This was on a 15% SGP+(2.0)/85% Sugarcane Top and Protien Concentrate diet (whereby the Protein Concentrate was decreased). The SGP+(2.0) was made using Sugarcane Bagasse from India, which has a normalized lignin concentration averaging 19%.
- Furthermore, Deer Run ranch continues to document with photographs expansive milk bags, colostrum rich in color and texture dripping from the bags prior to the calf dropping, and healthier/livelier calves (with reduced miscarriage and infant mortality).

- **General Health:** No red flags were seen in the data regarding health. The cows' manure doesn't show signs of nutrient deficiency or excess that could harm them:
  - **Protein:** Adequate (not deficient, since requirements met; not excessive, since only small surplus in manure).
  - **Phosphorus:** Borderline adequate - might consider a mineral supplement if not already provided, just to be safe, but one cow showed even surplus P, so they're not critically low as a herd.
  - **Energy:** Just meeting needs, which means cows are **maintaining condition but not gaining.** In a cow-calf operation, maintaining a BCS 6 in early lactation is actually a positive result (better than losing weight/condition).
  - **Manure Consistency:** Likely firm due to fiber - no scours (diarrhea) noted. Firm manure typically means the cows are not suffering from any digestive upsets. It might be a bit dry, but that's expected with high fiber. Ensuring they have plenty of water to drink is important with such a fibrous diet.
  - Dr. Robert Wells, formerly of the Nobel Research Institute and now Professor of the Practice and Paul C. Genho Endowed Chair in Ranch Management, King Ranch Institute (https://krirm.tamuk.edu/robert-wells-ph-d-pas/) has developed a system by which "Manure scoring determines supplementation needs" (https://www.noble.org/regenerative-agriculture/livestock/manure-scoring-determines-supplementation-needs/).
  - In this system, a Score 3 Manure Pat has been shown to contain: "12-15% CP; 62-70% TDN of diet."

- "Manure score 3 is ideal and will typically start to take on a normal pat form. The consistency will be similar to thick pancake batter. It will exhibit a slight divot in the middle. The pat will be deeper than a score 2 pat, but will not stack. This diet is not lacking nutritionally, yet is not in excess for the cow and her physiological stage."
- "Score 4 manure is thick and starting to become somewhat deeper, yet is not stacking. The consistency of the manure will be equivalent to peanut butter. This manure indicates a lack of degradable rumen protein, excess low quality fiber or not enough carbohydrates in the diet. Supplementation of additional protein with high rumen-degradable protein can increase total diet digestibility. Cottonseed meal and soybean meal are excellent sources of this type of protein."
- Dr. Wells provides guidance on nutritional adjustments that can be made in bovine ration to move the herd from under-performing nutritional mixes to optimized ration mixes.

In summary, the health indicators suggest **cows are doing well** on this bagasse/DDG feed. They're holding weight, staying in good condition, producing a reasonable amount of milk, and their manure looks normal for a high-fiber diet. There's **no sign of health problems** like weight loss, poor condition, or major nutrient imbalances.

- Again, SGP+™ uses Sugarcane Bagasse as one of several ingredients.

Comparison of the Three Samples

All three manure samples came from cows on the **same feed** and in the same herd. We'd expect similar results, and indeed the findings were **mostly consistent** with a bit of natural variation. Here's a comparison of key metrics across **Sample 168703, 168704, and 168705:**

Deer Run Cattle Feed Test - Comparison of Samples

Metric	Sample 168703	Sample 168704	Sample 168705
Cow Weight (lbs)	~1260lbs	~1250lbs	~1250lbs
Body Condition Score (1-9)	6.0 (Good)	6.0 (Good)	6.0 (Good)

Feed Crude Protein (% **of diet)**	~9.6%	~9%	~8%
Digestible Organic Matter(%)	~49-50%	~50%	~52%
DOM/CP Ratio	~ 7.7 (Slightly high)	~8.4 (High)	~10.4 (Very high)
Distillers Grains Fed (lbs/day)	5.8 lbs/day	5.8 lbs/day	5.8 lbs/day
Total Dry Matter Intake (lbs/day)	~29.2 lbs/day	~28-29 lbs/day	~29-30 lbs/day
Protein Intake vs Requirement	2.78 vs 2.54 lbs (+0.24 surplus)	~2.7 vs 2.5 lbs (+0.2 surplus)	~2.5 vs 2.4 lbs (Met requirement)
Predicted Weight Change	-0.02 lbs/day (stable)	0.0 lbs/day (stable)	~0.0 lbs/day (stable)
Milk (Potential vs Actual)	18.6 vs 14.4 lbs/day	~18 vs ~14 lbs/day	~18 vs ~15 lbs/day
Manure Output (Dry lbs/day)	12.2 lbs	~13lbs	~12lbs
Manure Nitrogen {% of OM)	2.31% (High)	1.29% (Moderate)	1.25% (Moderate)
Manure N Excreted (lbs/day)	~0.26 lbs/day	~0.15 lbs/day	~0.15 lbs/day
Manure Phosphorus (% **of OM)**	0.21%	0.32%	0.27%
Manure P Excreted (lbs/day)	~0.03 lbs/day	~0.04 lbs/day	~0.03 lbs/day

What's Consistent: All three cows ate about the same amount, got the same supplement, and stayed in good shape. Protein was sufficient in each case, and energy was the limiting factor for all. Each cow's manure output was around 12-13 lbs DM with similar fiber content. They all maintained weight with no gain or loss.

What's Different: There were slight variations in the manure nutrient content:

- Sample **168703** had a bit **higher protein in the forage portion** of the diet (6.4% vs ~5% in the others) and ended up excreting more nitrogen in manure. This might mean that cow ate or absorbed protein a little differently (perhaps using slightly less for milk). Her manure P was the lowest (0.21%), hinting she might have been hanging onto more phosphorus or had a tad less in her diet portion.
- Sample **168704** showed the **highest phosphorus** in manure (0.32%), suggesting she had plenty of P (or didn't need as much for milk/bones) - essentially confirming dietary P was adequate. Her manure nitrogen was much lower than 168703's, indicating she was efficient with using protein. She also had the ratio of digestible matter to protein at the higher end (~8.4), suggesting the forage she ate was very low in protein.

- Sample **168705** had the **lowest forage protein (around 5%)** but the highest digestibility (52% DOM). This cow likely ate a very fiber-heavy mix that was slightly more digestible than the others (maybe a bit more of the DDG or more of any green pickings available). Her manure N and P were both moderate (1.25% N, 0.27% P), falling between the other two cows.

Despite these small differences, **all results are in the same ballpark.** In a real-world sense, if you're the ranch family comparing these three samples, you'd say: *"They're pretty darn similar - no wild outliers. One cow's manure had a bit more nitrogen, another had a bit more phosphorus, but overall the feed is affecting them all alike."*

Conclusion - Is the Bagasse/DDG Feed Effective?

Yes - the 80/20 Bagasse-DDG feed is holding up well. In plain terms, the cows are **maintaining their weight and body condition,** which is crucial for a herd with young calves. They're getting **enough protein** (thanks to the DDG) and just about enough energy from the bagasse to stay level. Milk production is decent on this diet, though not maxed out - which is expected because bagasse isn't a high-energy feed. The manure shows **nutrients are being utilized efficiently** (not too much wasted protein or mineral), and it also hints at some side perks like possibly less odor and fewer flies due to the drier, fibrous nature.

For a ranching family, these results indicate that **the new bagasse-based feed is doing its job:**

- The cows are **healthy and not losing weight.**
- **Nutritional needs are met,** with just a small protein cushion and adequate minerals (might keep an eye on phosphorus, but it's close to adequate).
- There's **no sign of digestive upset -** manure is normal for this kind of feed.
- **Environmental notes:** The nutrients in manure will go back to the pasture (nitrogen and phosphorus recycling), and there's a possibility of **lower fly pressure and manageable methane** levels given the nature of the manure and feed.

In summary, the bagasse/DDG TMR appears to be a **beneficial and cost-effective feed** for the Deer Run cows. It uses a cheap fibrous byproduct (bagasse) but is balanced with distillers grains so the cows stay in good shape. The three samples confirm consistent performance across the herd. This should give the family confidence that the new feed is **effective and safe** for their cattle, with the added bonus that it might help a bit with pasture nutrient return and pest control. It's always wise to keep monitoring, but as of this report, the **bagasse feed experiment looks like a success** for the ranch.

IFUS Addendum 1: a WIP (Work-In-Progress) – Map in detail the continuous biochemical pathway for S- / G- / & H- Lignin by respective microbe (e.g., White Rot Fungi, Brown Rot Fungi, Bacteria, etc.) into components that would enable and/or deter effective Bovine Herd Performance as indicated above.

1. How does degraded lignin affect two stage fermentation?
   1.1. Degraded lignin affects two-stage fermentation as follows:
      1.1.1. Lignin depolymerization: Lignin is decomposed into oligomers or monomers, which are then completely degraded into carbon dioxide, water, and minerals (2).
      1.1.2. De-methoxylation: During anaerobic degradation, de-methoxylation is the first step, followed by ring cleavage and fermentation into methane and carbon dioxide (3).
      1.1.3. Lignin can be degraded in nature through two stages: depolymerization and mineralization (4).
      1.1.4. Experiments on lignin biodegradation: Acidic and mild alkaline conditions were used, with hydrogen peroxide for acidic pretreatment and bacterial growth on Luria and Bertani medium with alkali lignin as the sole carbon source for alkaline biodegradation (5).
   1.2. What exactly does this content mean? Is this *in vitro*? Is this in synthetic degradation and depolymerization of lignin? Is this supporting biofuel production from lignin or clarifying effects on bovine digestion? Are there overlays?
      1.2.1. Ref. 2: "Biological degradation of lignin: A critical review on progress and perspectives," Lei Zhao, et.al., Industrial Crops and Products, Volume 188, Part B, 15 November 2022, 115715
      1.2.2. Ref. 3: "Lignin degradation under anaerobic digestion: Influence of lignin modifications -A review," Muhammad Usman Khan, et.al., Biomass and Bioenergy, Volume 128, September 2019, 105325
      1.2.3. Ref. 4: "Chapter 9 - Microbial degradation of lignin: conversion, application, and challenges," Aryama Raychaudhuri, et.al., Development in Wastewater Treatment Research and Processes, Innovative Microbe-Based Applications for Removal of Chemicals and Metals in Wastewater Treatment Plants, 2022, Pages 195-219
         1.2.3.1. NOTE: Bagasse known to capture heavy metals. Is bagasse a clathrate compound? If so, are the ionic minerals in Nutri-

Mastic easily absorbed into the bagasse fiber, then find themselves pushed into the cellular wall or something to that effect to aid in the accelerated degradation and depolymerization of lignin (while offer added digestive / absorptive support and/or an effect on the ruminal mitochondria or other cellular mechanisms?"

1.2.3.2. Ref. 5: Acidic Versus Alkaline Bacterial Degradation of Lignin Through Engineered Strain E. coli BL21(Lacc): Exploring the Differences in Chemical Structure, Morphology, and Degradation Products, Gabriel Murillo Morales, et.al., Front Bioeng Biotechnol. 2020 Jun 30;8:671. doi: 10.3389/fbioe.2020.00671.

2. Does lignin have fractal components?

2.1. Yes, lignin does have fractal components. Research indicates that lignin macromolecules exhibit fractal characteristics, particularly in their aggregation and structural organization. Specifically, lignin is classified as a fractal object, with studies showing it possesses a fractal dimension of approximately 2.7, indicating its complex and hierarchical structure (1 & 2)

2.1.1. Macromolecular Hydrodynamics and Fractal Structures of the Lignins of Fir Wood and Oat Husks, Anatoly Karmanov, et.al., Polymers (Basel). 2023 Sep 1;15(17):3624. doi: 10.3390/polym15173624

2.1.2. Lignin. Structural organisation and fractal properties, A.P. Karmanov, et.al., Russian Chemical Reviews 72 (8) 715±734 (2003), Russian Academy of Sciences and Turpion Ltd.

2.2. Lignin fraction with low molecular weight was favorable for depolymerization into syringyl and alkyl syringyl, while the high molecular weight lignin was prone to depolymerize into guaiacyl and alkyl guaiacyl products.

2.2.1. Effect of structural characteristics on the depolymerization of lignin into phenolic monomers, Cheng Chen, et.al, Fuel, Volume 223, 1 July 2018, Pages 366-372

2.2.2. Comparative study for the separation and depolymerization behavior of lignin from different separation methods, Bochao Yan, et.al., Fuel, Volume 358, Part B, 15 February 2024, 130145

2.2.3. Depolymerization of lignin: Recent progress towards value-added chemicals and biohydrogen production, Hina Ramzan, et.al., Bioresource Technology, Volume 386, October 2023, 129492

2.2.4. Lignin depolymerisation strategies: towards valuable chemicals and fuels, Chunping Xu, et.al., Chemical Society Reviews, Issue 22, 2014

2.2.5. Effects of solvents in the depolymerization of lignin into value-added products: a review, Raikwar, D., et.al., Biomass Conv. Bioref. 13, 11383–11416 (2023). https://doi.org/10.1007/s13399-021-02030-7

2.2.6. The effects of lignin source and extraction on the composition and properties of biorefined depolymerization products, Natalia Obrzut, et.al., RSC Sustainability, Issue 9, 2023 https://pubs.rsc.org/en/content/articlelanding/2023/su/d3su00262d

2.2.7. Chemical Modification of Lignin by Polymerization and Depolymerization, Lopez-Camas, K., et.al. (2020). Chemical Modification of Lignin by Polymerization and Depolymerization. In: Sharma, S., Kumar, A. (eds) Lignin. Springer Series on Polymer and Composite Materials. Springer, Cham. https://doi.org/10.1007/978-3-030-40663-9_5

2.2.8. Effect of structural characteristics on the depolymerization of lignin into phenolic monomers, Cheng Chen, et.al., Fuel, Volume 223, 1 July 2018, Pages 366-372

2.3. S-lignin (sinapyl lignin) has a lower molecular weight than H-lignin (hemicellulose). Specifically, S-lignin is composed mainly of G-units, while H-lignin contains G, S, and H units, leading to a higher molecular weight for H-lignin. The molecular weight of lignin can vary widely depending on the source and processing methods, but generally, S-lignin is considered to have a lower molecular weight than H-lignin.

2.3.1. Lignin fractionation: Effective strategy to reduce molecule weight dependent heterogeneity for upgraded lignin valorization, Tairan Pang, et.al., Industrial Crops and Products, Volume 165, July 2021, 113442

2.3.2. Comparison of lignin distribution, structure, and morphology in wheat straw and wood, Liming Zhang, Industrial Crops and Products, Volume 187, Part B, 1 November 2022, 115432

2.3.3. 4. Techniques for Characterizing Lignin, Nicole M. Stark, et.al., https://www.fpl.fs.usda.gov/documnts/pdf2016/fpl_2016_stark001.pdf

2.4. Does lower molecular weight S-lignin affect the morphology of lignin?

2.4.1. Does affect the morphology of lignin

2.4.2. Does lower molecular weight S-lignin affect the morphology of lignin?

2.4.3. Lower molecular weight S-lignin does affect the morphology of lignin. The size of lignin nanoparticles (LNPs) decreases with increasing molecular weight (Mw) of the lignin fraction. This is due to a decrease in the number of phenolic hydroxyls and an increase in the number of aliphatic hydroxyl units in the lignin fraction. The diameter of the LNP's can vary between 80 and 500 nm, depending on the Mw of the initial lignin and its concentration. The number of methoxy and phenolic groups in the aromatic ring, the aliphatic hydroxyls and β-O-4 bonds in side chains, and the type of lignin phenylpropanoid units all significantly affect the morphology and surface structure of the LNPs. This indicates that the inherent properties of the lignin from which the LNPs are derived are crucial for the controlled design and synthesis of LNPs, which in turn affects their properties and applications. The Royal Society of Chemistry

2.4.3.1. Structural and molecular-weight-dependency in the formation of lignin nanoparticles from fractionated soft- and hardwood lignins, Ievgen V. Pylypchuk, et.al., DOI: 10.1039/D0GC04058D (Paper) Green Chem., 2021, 23, 3061-3072,

https://pubs.rsc.org/en/content/articlehtml/2021/gc/d0gc04058d

2.4.4. Morphological Properties of Lignin, Bouhfid, R., Qaiss, A.E.K., Raji, M. (2025). Morphological Properties of Lignin. In: Jawaid, M., Ahmad, A., Meraj, A. (eds) Handbook of Lignin. Springer, Singapore. Exploring the Effects of Different Cross-Linkers on Lignin-Based Thermoset Properties and MorphologiesDoes S-lignin crosslink more than H-lignin

2.4.4.1. Lignin, the Lignification Process, and Advanced, Lignin-Based Materials, Maria Balk, et.al., Int J Mol Sci. 2023 Jul 19;24(14):11668. doi: 10.3390/ijms241411668

2.4.4.2. Exploring the Effects of Different Cross-Linkers on Lignin-Based Thermoset Properties and Morphologies, Iuliana Ribca, et.al., ACS Sustainable Chemistry & Engineering, Vol 9/Issue 4

2.4.4.3. Covalent interactions between lignin and hemicelluloses in plant secondary cell walls, Oliver M Terrett, et.al., Current Opinion in Biotechnology, Volume 56, April 2019, Pages 97-104

2.4.4.4. Hu, Z., Zhang, G., Muhammad, A. et al. Genetic loci simultaneously controlling lignin monomers and biomass digestibility of rice straw. Sci Rep 8, 3636 (2018). https://doi.org/10.1038/s41598-018-21741-y

TO BE CONTINUED