Part 2: Plausible Scientific Evidence of the Efficacy of SGP+™ in Bovine Herd Performance through Bovine Ration Management

The Science Behind Mastic and Carob Individually and Collectively as well as Synergies with Ionic Minerals and Degraded/Depolymerized Lignin

(Continued from Part 1: Lignin, Degraded and Depolymerized Lignin, and Select Synergies from Mastic, Ionic Minerals, and Carob)

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One may entertain what is plausible by first entertaining that it is possible. In doing so, what is possible then may well become probable…and subsequently normal.

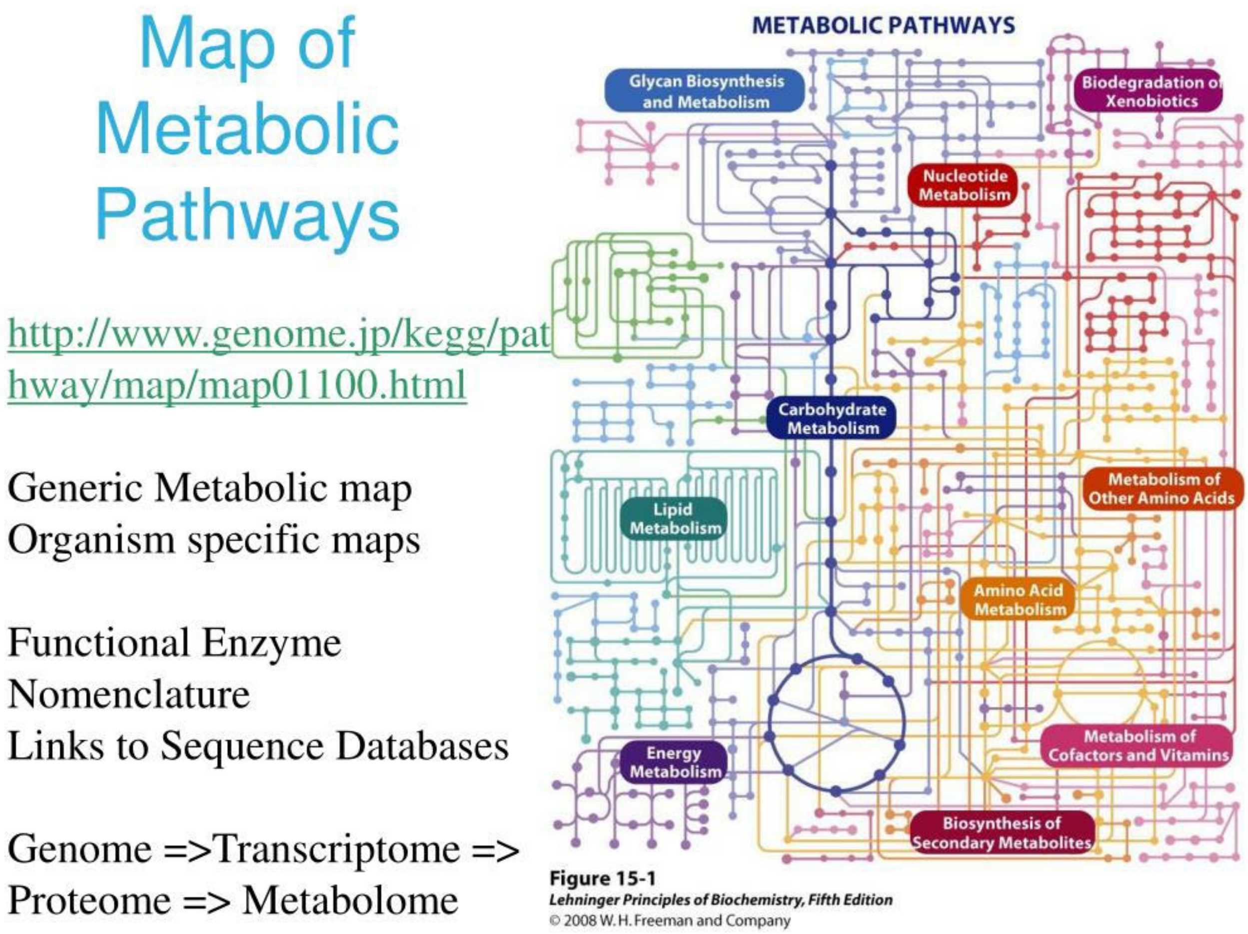
The information in this White Paper is continued in Part 2a and Part 2b White Papers. We do so as the science can be quite complex, especially when critical overlaps in the phytochemical make-up of the active ingredients of SGP+™ is explored. We also wish to note that these White Papers are repositories for the science we continue to uncover. Hence, these are considered "Works-In-Progress."

We begin with a reminder: 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.

In an attempt to offer plausible scientific evidence of the efficacy of SGP+™ in Bovine Herd Performance through Ration Management, we ask that you consider that in Part 1 of this paper, IFUS focused on but two or so Metabolic Pathways to suggest the impact of degraded and depolymerized lignin per IFUS Point 1c:

> IFUS Point 1c: The diagram below "Map of Metabolic Pathways" reflects the complexity that 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, we begin with a view of the myriad of

metabolic cycles, while noting that Part 2 will attempt to target selected cycles and their synergistic interconnections to other cycles.



Furthermore, IFUS will attempt to offer plausible explanation as to Bovine Herd Performance claims made by ranchers and dairymen applying SGP+™ as part of their respective Ration Management. Hence, a deeper and broader dive into the science is undertaken to offer said explanations as to ingredients in SGP+™ and how these ingredients:

(1) assist in the degradation and depolymerization of lignin in cooperation with the natural biome within Sugarcane Bagasse.

(2) contribute to the nutritional efficacy of SGP+™ through Crude Protein and Total Digestive Nutrients.

(3) provide ample energy to support, sustain, and improve Herd Performance Outcomes.

(4) improve bovine health.

(5) sequester, ameliorate, and/or eliminate unwanted CH_4 production.

(6) provide ample hydration and possibly *in vivo* cooling of the bovine.

(7) reduce fly and parasite infestations in pastures and herds.

(8) aid in soil replenishment.

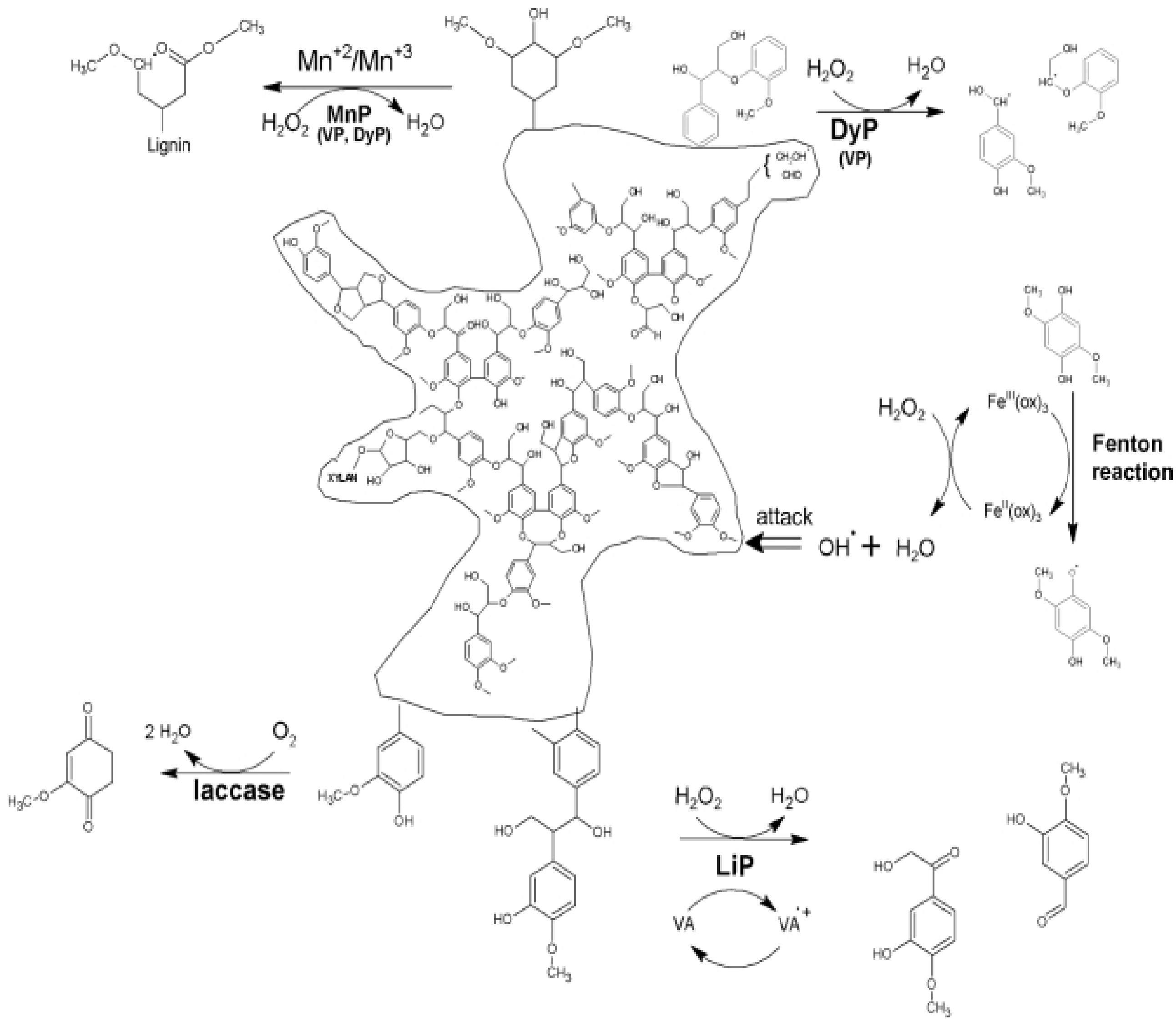
The science is offering evidence that bovine ruminal mitochondria (and possibly cellular mitochondria) may in fact be affected in such a way as to generate plausible explanations as to the claims made by ranchers and dairymen applying SGP+™ as part of their respective Ration Management.

Let's take a moment to recall IFUS Point 5f(2):

IFUS Point 5f(2): 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.

Figures 1, 2, 3, and 4 progressively illustrate lignin degradation and depolymerization pathways by microbes contained in Sugarcane Bagasse resulting in methane production sequestration through the production of water *in* vivo through ruminal mitochondria coupled with white rot fungi mitochondria as reflected in these particular pathways where lignin is involved in bovine digestion.

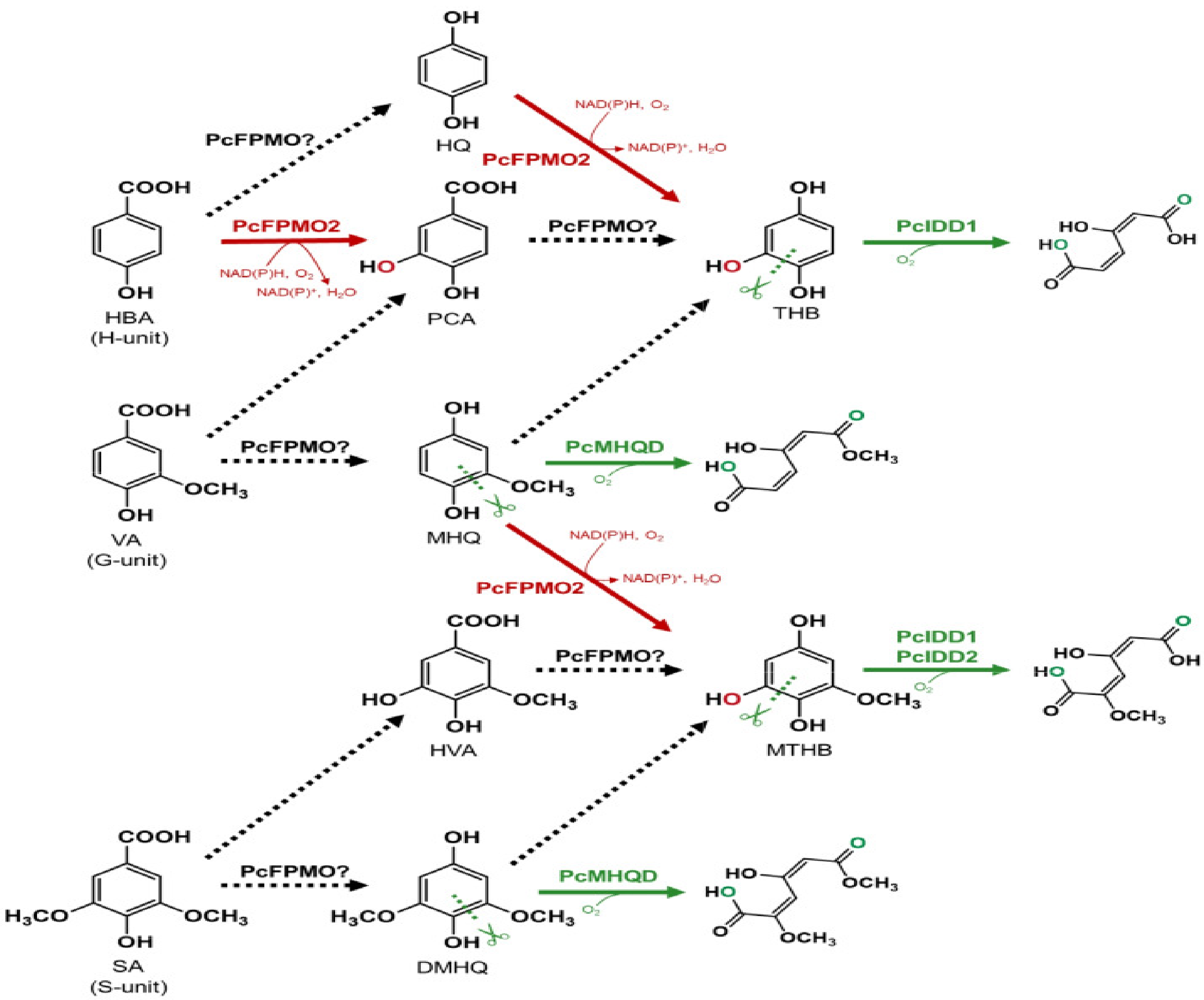
Fig.1



Note: The Mn and Fe Heme Peroxidases, laccase, the LiP (Ligin Peroxidase), the DyP (Dye-decolorization Peroxidase) producing water from their interactions with the lignin polymeric chain.

Note: Studies indicate that these types of interactions within *in vivo* bovine digestion scavenge free H+ so as to prevent CH4 production, while actually increasing ruminal efficiency. ADD REFERENCE

Fig. 2.



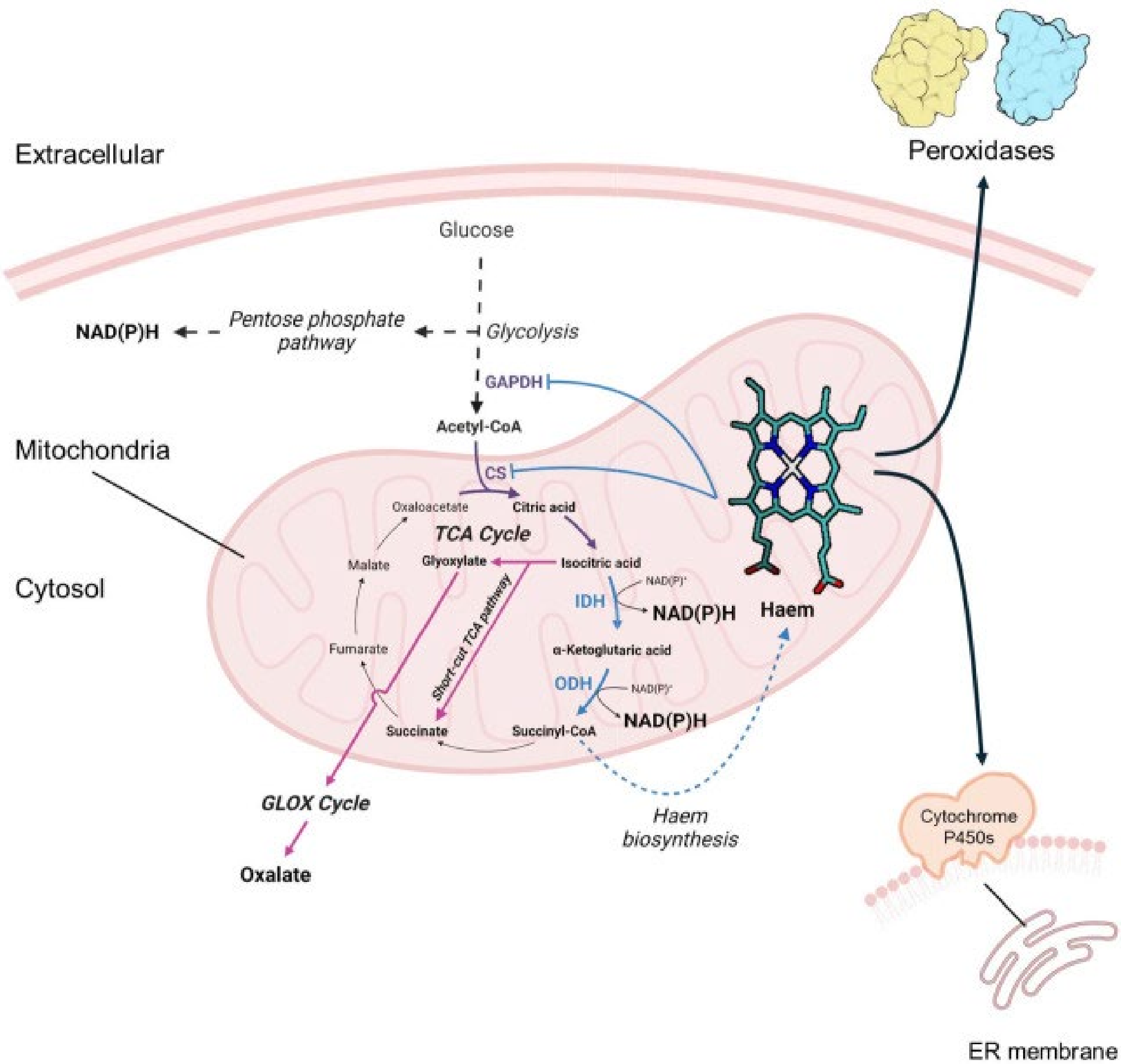
Metabolic pathways of HBA, VA, and SA in white-rot fungus *P. chrysosporium*. Dotted arrows indicate the estimated reactions, and solid arrows indicate the reactions by identified enzymes including flavoprotein monooxygenase 2 (PcFPMO2), MHQ dioxygenases (PcMHQD), intradiol dioxygenase 1 (PcIDD1), and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

Note: Lignin hydroxyphenyl units (H-unit) such as HBN and p-hydroxybenzoic acid (HBA)

Note: Lignin guaiacyl units (G-unit) such as VN and vanillic acid (VA)

Note: Lignin syringyl units (S-unit) including SN and syringic acid (SA)

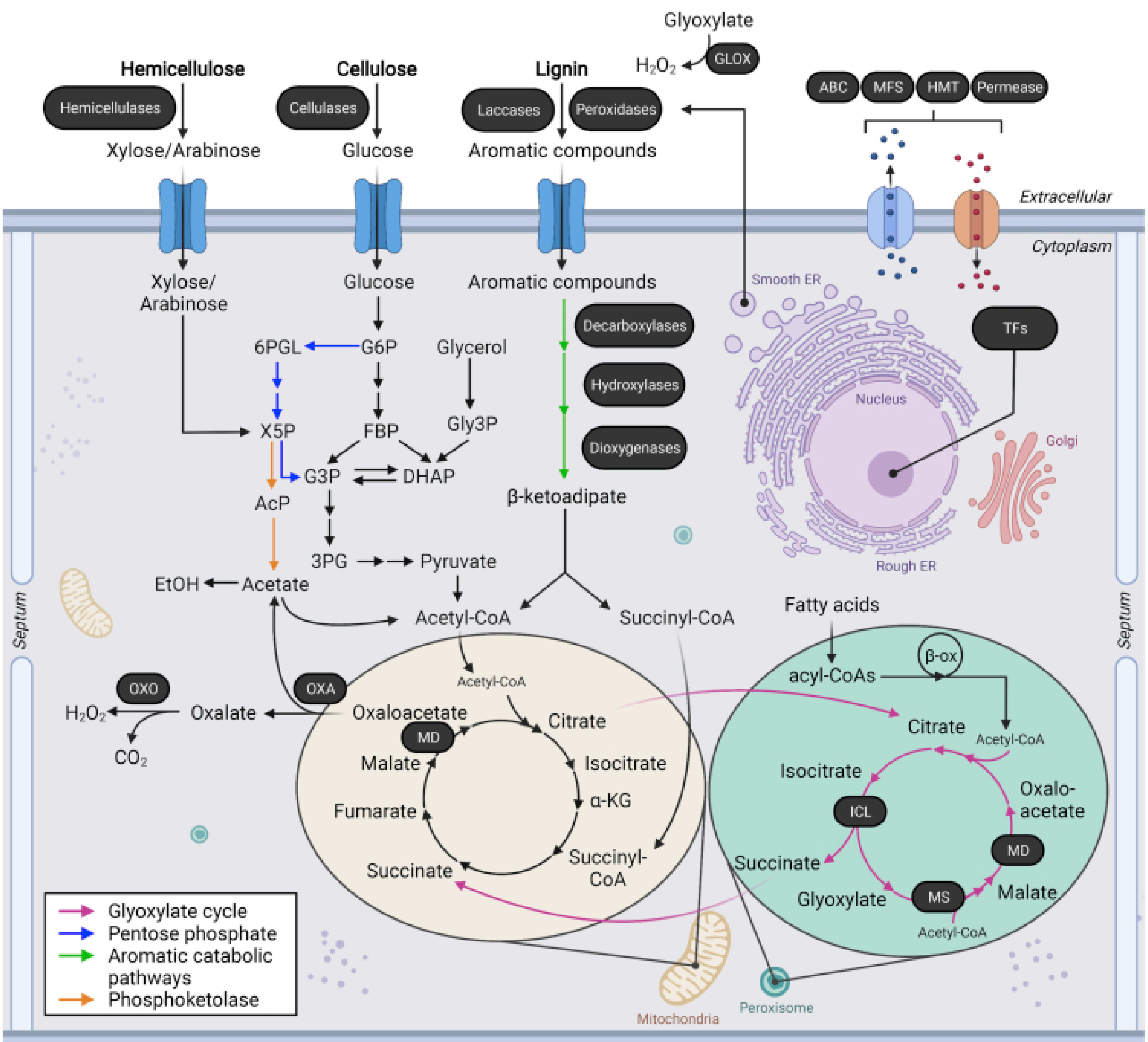
Fig. 3.



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)

Note: Haem(British) is the same as Heme.

Fig. 4



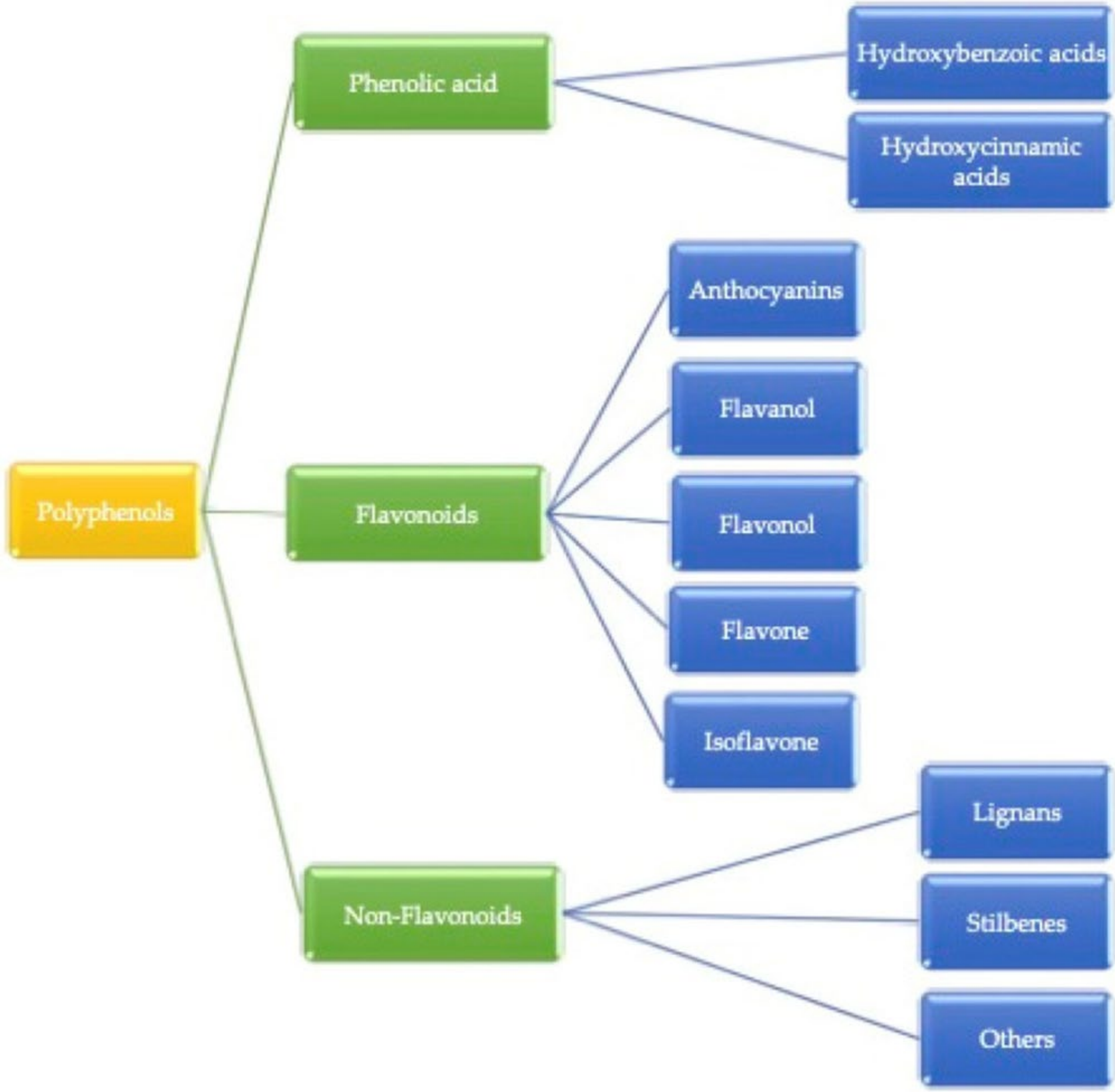
Summary of proposed intracellular catabolic pathways during lignocellulose degradation in WRF.

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.

Pathways of this nature beg the question as to whether nature mimics pathways from one organism to another.

Furthermore, in our exploration into the ingredients of SGP+™ (Carob, Chios Mastic Gum, and Sugarcane Bagasse/SB (specifically the lignin degraded and depolymerized by the natural biome contained in the SB), we beg the question as to beneficial aspects of each ingredient shared across all three. What we find in common is partially illustrated below, that being the plant-based polyphenols that are then segregated into families of compounds,

Our exploration leads us into understand these compounds from an individual, collective, and integrated perspective…especially from the perspective of impacts on beef and dairy cow performance.



(ADD SOURCE)

Hence, one can plausibly entertain that lignin degradation and subsequent depolymerization initiated by the natural biome existing within Sugarcane Bagasse is accelerated by applying Mastic, Ionic Minerals, Water, and Carob, and processed in a proprietary method that shifts the steaming Sugarcane Bagasse (an ND-40 Fiber) into SGP+™ as indicated by a decrease in material temperature from approximately 145 degrees F to approximately 94 degrees F in less than 2-minutes with continued cooling of a 2000-lb bale to approximately 84-degrees F.

Then, when said product (SGP+™) is applied as part of Bovine Ration, ranchers and dairymen report a myriad of improvements in Herd Performance. How can that be possible?

There are adages that tell us that a broken clock is correct twice a day and even a blind squirrel can every now and then chase an acorn up a tree. To that point, the IFUS Scientific Team often sees itself as both a broken clock and blind squirrel in its pursuit to understand the efficacy of SGP+™ as reported by ranchers and dairymen applying the product as a technology in Bovine Ration Management. Hence, the continued search of that acorn and the correct time, which may suggest that less artificial interference from human-kind and a return to natural processes may in fact be the path SGP+™ is providing to us.

Hence, with the aforementioned review complete, let us now consider the following inquiries:

1. **<u>Does SGP+™ play a role in natural cellular reprogramming at the mitochondrial level?</u>**

1.1 We are beginning to wonder if SGP+™ as well as SupremeAG™ may in fact be supporting through natural means "cellular reprogramming."

1.2 If you review the overall 70-plus pages of science we now offer as evidence of the efficacy of SGP+™ in Bovine Nutrition (Part 1), you will find the production of certain chemicals at the mitochondrial level. However, we are wondering if that production is also being stimulated and supported by SGP+™.

1.3 In "Remodeling of mitochondrial morphology and function: An emerging hallmark of cellular reprogramming," Dr. Anuj Rastogi of Vanderbilt University, et.al. states, "Recent studies suggest an emerging landscape in which mitochondrial morphology and function have an active role in maintaining and regulating changes in cell identity." (Stress. 2019 May 27;3(6):181–194. doi: 10.15698/cst2019.06.189)

1.4 This leads us to wonder as to why, or more so how, these metabolic pathways are affecting Bovine Herd Performance though the application of SGP+™ in respective Ration Management strategies.

1.4.1 One plausible explanation is "heme" as a native protein, yet more so as peroxidase, specifically Mn and Fe. When one considers the kinetics at the molecular cellular level from the vantage of the "transition state", then one finds plausible science in the management of said "transition state" by catalysts that provide the Delta S required for a specific "product" to be produced. If memory serves us correctly, this science was illuminated by Dr. Stryer at Stanford and Dr. Byers at Tulane on ground-breaking research on the kinetics of the transition state of the Hyaluronidase Enzyme System in the production of Hyaluronic Acid so to improve the efficacy of cellular stabilization resulting in the prevention of metastasis in cancer cells.

1.5 Let us remember that, "Peroxidases or peroxide reductases (EC number 1.11.1.x) are a large group of enzymes which play a role in various biological processes. They are named after the fact that they commonly break up peroxides, and should not be confused with other enzymes that produce peroxide, which are often oxidases."

1.6 Also, Dr. Daniel J Simpson of the University of Edinburgh, et.al., states in a study titled, "Cellular reprogramming and epigenetic rejuvenation, (Clin Epigenetics. 2021 Sep 6;13:170. doi: 10.1186/s13148-021-01158-7) that, "Recent achievements, such as the generation of induced pluripotent stem cells, have delivered preliminary evidence that slowing down and reversing the ageing process might be possible. However, these techniques usually involve complete dedifferentiation, i.e. somatic cell identity is lost as cells are converted to a pluripotent state. Separating the rejuvenative properties of reprogramming from dedifferentiation is a promising prospect, termed epigenetic rejuvenation. Reprogramming-induced rejuvenation strategies currently involve using Yamanaka factors (typically transiently expressed to prevent full dedifferentiation) and are promising candidates to safely reduce biological age."

1.7 When overlaying this kind of science with the biochemistry of Mastic, Carob, Ionic Minerals, depolymerized Lignin, and the resulting products produced by microbes naturally occurring in Sugarcane Bagasse, one finds another plausible series of synergies that would suggest that SGP+™ is restoring the cellular structure of the bovine (specifically ruminal mitochondria) in a manner in which "Nature" intended…by reversing the impact of all that synthetic crap placed into the bovines diet combined with stripping the soil of nutrients (Humic Substances) and probiotic microbes, as well as the myriad of synthetic chemicals (to include drugs) used to create a supposedly healthier cow.

1.8 We are wondering if SGP+™ is actually creating a natural form of "mitochondrial eugenics", by reversing the damage done and returning the bovine to a "natural stasis."

1.9 Is this merely science-fiction or plausible science-faction? Hence, the IFUS Scientific Team has dived deeper into the myriad of scientific studies being performed and published.

2 <u>As an example, an overlay considering a "Metabolic Pathway":</u>

2.1 "TNF-α is a pro-inflammatory cytokine produced primarily by macrophages and T cells in response to infection or tissue damage. It serves critical roles, including: (1) Activating the immune system to fight pathogens, (2) Regulating apoptosis (programmed cell death). (3) Mediating systemic inflammation.

2.1.1 However, overproduction of TNF-α can result in uncontrolled inflammation, tissue damage, and the progression of various diseases.

2.1.2 In bovines, the effect of TNF-α has been documented:

2.1.2.1 "Lameness is a common condition in dairy cattle caused by infectious or noninfectious agents. Joint lesions are the second most common cause of lameness and can be diagnosed in association with the presentation of digit injuries. Fibroblast-like synoviocyte (FLS) are predominant cells of synovia and play a key role in the pathophysiology of joint diseases, thus increasing the expression of proinflammatory mediators. Tumor necrosis factor-alpha (TNF-α) is a potent proinflammatory cytokine involved in cyclooxygenase 2 (COX-2) and proinflammatory cytokine expression in FLS.

Previously, TNF-α was demonstrated to increase hypoxia-inducible Factor 1 (HIF-1), a transcription factor that rewires cellular metabolism and increases the expression of interleukin (IL)-6 in bovine FLS (bFLS).” (Bovine tumor necrosis factor-alpha Increases IL-6, IL-8, and PGE2 in bovine fibroblast-like synoviocytes by metabolic reprogramming, Carolina Manosalva, et.al., Sci Rep. 2023 Feb 24;13:3257. doi: 10.1038/s41598-023-29851-y

2.1.2.2 “Refractory diseases, including bacterial infections, are causing huge economic losses in dairy farming. Despite efforts to prevent and treat those diseases in cattle, including the use of antimicrobials, it is not well controlled in the field. Several inflammatory cytokines, including tumor necrosis factor alpha (TNF-α), play important roles in disease progression; thus, blocking these cytokines can attenuate the acute and sever inflammation and may be a novel strategy for treatment. However, biological drugs targeting inflammatory cytokines have not been used in cattle. Therefore, in this study, bovine sTNFR1 and sTNFR2 IgG1 Fc-fusion proteins (TNFR1-Ig and TNFR2-Ig) were produced, and their anti-inflammatory functions were analyzed in vitro, to develop decoy receptors for bovine TNF-α.” Effects of bovine tumor necrosis factor alpha decoy receptors on cell death and inflammatory cytokine kinetics: potential for bovine inflammation therapy, Sotaro Fujisawa, et.al., BMC Vet Res. 2019 Feb 28;15:68. doi: 10.1186/s12917-019-1813-0.

2.1.2.3 Associative data and some controlled studies suggest that the inflammatory cytokine tumor necrosis factor (TNF) α can induce fatty liver in dairy cattle. However, research demonstrating that TNFα is a necessary component in the etiology of bovine fatty liver is lacking. The aim of this work was to evaluate whether blocking TNFα signaling with a synthetic cyclic peptide (TNF receptor loop peptide; TRLP) would improve liver metabolic function and reduce triglyceride accumulation during feed restriction. Effects of TNF receptor blockade on in vitro cell survival and response to negative energy balance in dairy cattle, C A Martel, et.al., Effects of TNF receptor blockade on in vitro cell survival and response to negative energy balance in dairy cattle, J Anim Sci Biotechnol. 2018 Jan 10;9:6. doi: 10.1186/s40104-017-0224-y\

2.1.2.4 Tumor necrosis factor-α promotes lipolysis and reduces insulin sensitivity by activating nuclear factor kappa B and c-Jun N-terminal kinase in primary bovine adipocytes, Xiliang Du , et.al., J Anim Sci Biotechnol. 2018 Jan 10;9:6. doi: 10.1186/s40104-017-0224-y

2.2 The Phenolic Acid known as Gallic acid is a compound found in and/or produced by all three of the active ingredients in SGP+™ (Carob, Chios Mastic Gum, and Sugar Cane Bagasse).

2.2.1 Gallic acid (also known as 3,4,5-trihydroxybenzoic acid) is a trihydroxybenzoic acid with the formula C6H2(OH)3CO2H. It is classified as a phenolic acid. It is found in gallnuts, sumac, witch hazel, tea leaves, oak bark, and other plants.[1]

2.2.1.1 Ref.(1) Haslam, E.; Cai, Y. (1994). "Plant polyphenols (vegetable tannins): Gallic acid metabolism". Natural Product Reports. 11 (1): 41–66. doi:10.1039/NP9941100041. PMID 15206456.

2.2.2 Gallic acid affects TNF-α through necroptosis and induces oxidative stress by suppressing catalase activity and depleting glutathione (1,2). It promotes IL-6 release and inhibits TNF-α release of neutrophils (3). Gallic acid and its esters reduce TNF-α-induced NFκB activation(4,5).

2.2.2.1 Ref. 1 & 2: “The beneficial effect of GA on the reduction of animal hepatofibrosis has been indicated due to its antioxidative property.

However, the cytotoxicity of GA autoxidation causing cell death has also been reported. Herein, we postulated that GA might target activated hepatic stellate cells (aHSCs), the cell type responsible for hepatofibrosis, to mitigate the process of fibrosis." "Collectively, this is the first report indicating that GA induces TNF signaling-triggered necroptosis in aHSCs, which may offer an alternative strategy for the amelioration of liver fibrosis." Gallic acid induces necroptosis via TNF-α signaling pathway in activated hepatic stellate cells, Ya Ju Chang, et.al., PLoS One. 2015 Mar 27;10(3):e0120713. doi: 10.1371/journal.pone.0120713. eCollection 2015.

2.2.2.2 Ref. 3: Influence of gallic acid on porcine neutrophils phosphodiesterase 4, IL-6, TNF-α and rat arthritis model, Dai-xun JIANG, et.al., Journal of Integrative Agriculture, Volume 14, Issue 4, April 2015, Pages 758-764

2.2.2.3 REF. 4 & 5: Suppression of TNF-α induced NFκB activity by gallic acid and its semi-synthetic alkyl-gallates: Possible role in cancer chemoprevention, Mauro C C Morais, et.al., Nat Prod Res. 2010 Nov;24(18):1758-65. doi: 10.1080/14786410903335232.

2.3 Furthermore, "Heme oxygenase (HO)-1, an enzyme involved in heme catabolism, is upregulated in oxidant and inflammatory settings. Recent work suggests that HO-1 induction may serve a protective function against oxidant injury. Overexpressing HO-1 mitigated the TNF-alpha-mediated changes in cell cycle progression and apoptosis, perhaps by decreasing COX activity (2,3,5)."

2.3.1 Ref. 2: Role of human heme oxygenase-1 in attenuating TNF-alpha-mediated inflammation injury in endothelial cells, Taketoshi Kushida, et.al., J Cell Biochem. 2002;87(4):377-85. doi: 10.1002/jcb.10316.

2.3.2 Ref. 3: Effect of tumor necrosis factor-alpha and interleukin-1 alpha on heme oxygenase-1 expression in human endothelial cells, C M Terry, et.al., Am J Physiol. 1998 Mar;274(3):H883-91. doi: 10.1152/ajpheart.1998.274.3.H883.

2.3.3 Ref. 5: Heme oxygenase-1 inhibits TNF-α-induced apoptosis in cultured fibroblasts, Irina Petrache, et.al., American Journal of Physiology-Lung Cellular and Molecular PhysiologyVol. 278, No. 2, 01 Feb 2000https://doi.org/10.1152/ajplung.2000.278.2.L312

2.4 Also, "Heme oxygenase (HO)-1 catalyzes the oxidative cleavage of heme to yield equimolar amounts of biliverdin, iron, and carbon monoxide. HO-1 is a stress response protein, the induction of which is associated with protection against oxidative stress. The mechanism(s) of protection is not completely elucidated, although it is suggested that one or more of the catalytic by-products provide antioxidant functions either directly or indirectly." Irina Petrache, et.al, American Journal of Physiology-Lung Cellular and Molecular Physiology Vol. 278, No. 2.

2.4.1 It is acknowledged that this focus is on "heme oxygenase". However, when one overlays this information onto the Heme Peroxidases of Mn and Fe, one finds that these particular Heme systems may in fact trigger regulatory response in the mitochondria by scavenging Free-Radicals…hence the preventive effect accentuated (peroxidase) with the negative effects attenuated (oxidase) into kinetic stasis.

2.4.2 Peroxidase enzymes can oxidize a multitude of substrates in diverse biological processes. According to the latest phylogenetic analysis, there are four major heme peroxidase superfamilies. In this review, we focus on certain members of the cyclooxygenase-peroxidase superfamily (also labeled as animal heme peroxidases) and their connection to specific NADPH oxidase enzymes which provide H_2O_2 for the one- and two-electron oxidation of various peroxidase substrates. The family of NADPH oxidases is a group of enzymes dedicated to the production of superoxide and hydrogen peroxide. There is a handful of known and important physiological functions where one of the seven known human NADPH oxidases plays an essential role. In most of these functions NADPH oxidases provide H_2O_2 for specific heme peroxidases and the concerted action of the two enzymes is indispensable for the accomplishment of the biological function. We discuss human and other metazoan examples of such cooperation between oxidases and peroxidases and analyze the biological importance of their functional interaction. We also review those oxidases and peroxidases where this kind of partnership has not been identified yet., The Relationship of NADPH Oxidases and Heme Peroxidases: Fallin' in and Out, Gábor Sirokmány, et.al., Front Immunol. 2019 Mar 5; 10:394. doi: 10.3389/fimmu.2019.00394

2.4.3 This series of studies (a trip down a rabbit hole from one perspective), begs the question: Mere coincidence or plausible explanations as to the nature of biochemistry being produced by SGP+™. Furthermore, the studies reflect the potential of a reprogramming of the biochemistry at the mitochondrial level, that may may in fact become a reprogramming of cellular function.

2.5 Dr. Pauling might suggest were he alive that this type of science ultimately manifests at the atomic and sub-atomic level resulting in the reconstruction of damaged Mitochondrial DNA. Hence, a truly regenerated cell through cellular reprogramming results, specifically at the ruminal mitochondrial level. (See Item #3).

3 **<u>Dr. Pauling, Vitamin C, and Gallic Acid (an active ingredient found in Mastic and Carob as well as a byproduct of depolymerized Lignin)</u>**

3.1 Dr. Linus Pauling suggested that Vitamin C had efficacy in treating any number of human (and mammalian) illnesses.

3.1.1 In many ways, Dr. Pauling's reasoning met with scientific scrutiny and in some ways derision. However, new studies indicate that Dr. Pauling's reasoning may have at the time leaped beyond the limitation of that period's scientific thinking, reasoning, and capability. As another example of an overlay, specifically in consideration of Gallic Acid (a compound found in Mastic and Carob as well as one produced by the depolymerization of Sugarcane Bagasse lignin by naturally occurring microbes), one finds studies like the following:

3.1.1.1 "Vitamin C and Gallic Acid Ameliorate Motor Dysfunction, Cognitive Deficits, and Brain Oxidative Stress in a Valproic Acid-Induced Model of Autism," Parnia Tarahomi, et.al., Brain Behav. 2025 Feb 5;15(2):e70262. doi: 10.1002/brb3.70262.

3.1.2 Furthermore, "Research on antioxidant performance of diglucosyl gallic acid and its application in emulsion cosmetics," Kai-Qiang Zhang, et.al, Int J Cosmet Sci. 2022 Apr;44(2):177-188. doi: 10.1111/ics.12766. Epub 2022 Mar 15, states, "Diglucosyl gallic acid is a whitening active with powerful whitening function. When it acts on human skin, microorganisms on the skin surface and part of the stratum corneum produce α-glucosidase to sever the glucose bond of diglucosyl gallic acid, thereby converting part of diglucosyl gallic acid into gallic acid, acting on the skin and exerting the excellent effects of diglucosyl gallic acid and gallic acid at the same time. Diglucosyl gallic acid has high stability and water solubility, it can reduce free radical generation, inhibit tyrosinase generation, prevent melanin transfer, and control skin inflammation. The present study investigates the in vitro tyrosinase inhibition activity, antioxidant capacity of diglucosyl gallic acid as well as its clinical efficacy as a cosmetic ingredient."

3.1.3 Once again, the overlayed synergies continue to suggest that IFUS may be on the tip of the iceberg in creating a revolutionary set of products that can have invaluable effects on mammalian and plant life…a "Return to the Garden of Eden Effect" of sorts.

4 **Are we reaching or does the science continue to direct us towards cellular rejuvenation through cellular reprogramming?**

4.1 Considering the following study, which suggests, “The present review reports the main benefits of polyphenols in modulating mitochondrial processes that favor the regulation of energy expenditure and offer benefits in the management of obesity, especially thermogenesis and mitochondrial biogenesis. (Effects of Polyphenols on Thermogenesis and Mitochondrial Biogenesis, Tanila Wood dos Santos, et.al., Int J Mol Sci. 2018 Sep 13;19(9):2757. doi: 10.3390/ijms19092757)

4.1.1 Additionally, “The fungi of Lentinula edode LE16 and Pleurotus ostreatus PO45 were also found to degrade lignin of sugarcane bagasse by producing polyphenol oxidase (PPO) and Mn.” (Dong XQ, Yang JS, Zhu N, Wang ET, Yuan HL. Sugarcane bagasse degradation and characterization of three white-rot fungi. Bioresour Technol. 2013;131:443–451. doi: 10.1016/j.biortech.2012.12.182. [DOI] [PubMed] [Google Scholar])

4.2 To continue on this theme of synergistic and overlayed science, “The feeding of domestic animals with diets in which polyphenols are present is increasingly attracting the attention of nutritionists and scientists. This review summarizes the knowledge regarding polyphenols’ possible positive and negative effects and their bioavailability. The bioavailability of substances is a prerequisite for any post-absorption effect in vivo.” (Polyphenols in Ruminant Nutrition and Their Effects on Reproduction, Drago Bešlo, et.al., Antioxidants (Basel). 2022 May 14;11(5):970. doi: 10.3390/antiox11050970)

4.3 Furthermore, possible synergies found in studies like: “Polyphenol oxidase (PPO) is predominately associated with the detrimental effect of browning fruit and vegetables, however, interest within PPO containing forage crops (crops to be fed to animals) has grown since the browning reaction was associated with reduced nitrogen (N) losses in silo and the rumen.” (Forage polyphenol oxidase and ruminant livestock nutrition, Michael R F Lee, Front Plant Sci. 2014 Dec 8;5:694. doi: 10.3389/fpls.2014.00694)

4.4 This overlay continues with further supportive evidence: “There is growing interest in specialized metabolites for fortification strategies in feed and/or as an antioxidant, anti-inflammatory and antimicrobial alternative for the containment of disorders/pathologies that can also badly impact human nutrition. 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. Often conflicting results emerge from a review of the literature of recent years. However, the data suggest pursuing a deepening of the role of phenols and polyphenols in ruminant feeding, paying greater attention to the chemistry of the single compound or to that of the mixture of compounds more commonly used for investigative purposes." (Marialuisa Formato, et.al., Polyphenols for Livestock Feed: Sustainable Perspectives for Animal Husbandry? Molecules. 2022 Nov 10;27(22):7752. doi: 10.3390/molecules27227752)

4.5 As a reminder to the aforementioned content, we find more evidence in: "Polyphenols are secondary plant metabolites which have been shown to exert antioxidative and anti-inflammatory effects in cell culture, rodent and human studies. Based on the fact that conditions of oxidative stress and inflammation are highly relevant in farm animals, polyphenols are considered as promising feed additives in the nutrition of farm animals." (Potential of plant polyphenols to combat oxidative stress and inflammatory processes in farm animals, D. K. Gessner, et.al., Journal of Physiology and Animal Nutrition, Volume101, Issue4, August 2017, Pages 605-628)

4.6 Lastly, it is now believed in a review published by Dr. Lidija Jakobek and titled, "Interactions of polyphenols with carbohydrates, lipids and proteins," in Food Chemistry, Volume 175, 15 May 2015, Pages 556-567," that: (1) "Polyphenols interact with molecules like proteins, carbohydrates, lipids. (2) Interactions can result in creation of bigger associations. (3) Associations can change activities of polyphenols. (4) Understanding interactions will contribute to polyphenol bioactivity explanation."

5 **<u>Hence, we investigate polyphenols known as Gallotannins:</u>**

5.1 "Carob contains gallotannins, which are one of the main categories of phenolic compounds found in carob fruit (1 & 4). Carob bean gum, obtained from the seed endosperm of the carob tree, is also a galactomannan (2 & 3).

5.1.1. Ref 1: Functional polysaccharides of carob fruit: a review, Bao-Jie Zhu, et.al., Chin Med. 2019 Sep 30;14:40. doi: 10.1186/s13020-019-0261-x

5.1.2 Ref 4: Utilization of Carob Fruit as Sources of Phenolic Compounds with Antioxidant Potential: Extraction Optimization and Application in Food Models, Vlasios Goulas, Foods. 2019 Dec 24;9(1):20. doi: 10.3390/foods9010020

5.1.3. Ref.2: 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

5.1.4. Ref. 3: Functional polysaccharides of carob fruit: a review, Bao-Jie Zhu, et.al., Chin Med. 2019 Sep 30;14:40. doi: 10.1186/s13020-019-0261-x

5.2 Additionally, the gallotannins from carob are discussed in Table 1 found in "Chapter 6 - Nutraceutical and functional value of carob-based product: The LBG Sicilia Srl Case Study", Mario Testa, et.al., Case Studies on the Business of Nutraceuticals, Functional and Super Foods, Woodhead Publishing Series in Consumer Sci & Strat Market 2023, Pages 107-120

5.2.1 TABLE 1: Reviews related to physiological activities of gallotannins (GTs) documented in recent 5 years. (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)

Biological activity	Conclusion	References
Anti-inflammatory	Directly application of GTs containing preparations on inflamed tissues exhibit impact on inflammatory processes. However, structural changes mediated by gut microbiota during the transit through gastrointestinal tract should be referred. Coupled with dietary fiber, GTs may result in intestinal health benefits.	(7, 11)

Biological activity	Conclusion	References
Anti-bacteria	GTs inhibit growth of intestinal flora by preventing bacterial adhesion to the intestinal epithelium and prevent coliform diarrhea by inhibiting the bacterial enterotoxins and channels involved in the secretion of electrolytes and water into the lumen.	(12)
Anti-tumor	Represented by GTs, phytochemicals contained in *Mangifera indica*, *Punica granatum*, possess strong anti-tumor activity, inhibit breast cancer cell growth, proliferation, migration and invasion as well as trigger apoptosis and cell cycle arrest.	(13, 14)
Modulation of hemostasis system	Tannins, including GTs, modify the activity of platelets, coagulation, the fibrinolysis system, and endothelium, reduce the risk of thromboembolism.	(15)
Interference of metabolites	Due to the capacity to trap ammonia and H_2S, and to modify the composition and populations of the microbiota, tannins reduce the production of potentially toxic metabolites in farm animals, which would be benefit to the quality of animal products, i.e., organoleptic qualities of meat and milk.	(16)
Pharmacology and toxicology	Main pharmacological activities as well as toxicity of tannins were discussed.	(4)

5.2.2 Ref 7: Kiss AK, Piwowarski JP. Ellagitannins, gallotannins and their metabolites- the contribution to the anti-inflammatory effect of food products and medicinal plants. Curr Med Chem. (2018) 25:4946–67. 10.2174/0929867323666160919111559 [DOI] [PubMed] [Google Scholar]

5.2.3 Ref 11: Kim H, Castellon-Chicas MJ, Arbizu S, Talcott ST, Drury NL, Smith S, et al. Mango (Mangifera indica L.) polyphenols: anti-inflammatory intestinal microbial health benefits, and associated mechanisms of actions. Molecules. (2021) 26:2732. 10.3390/molecules26092732 [DOI] [PMC free article] [PubMed] [Google Scholar]

5.2.4 Ref 12: Girard M, Bee G. Invited review: tannins as a potential alternative to antibiotics to prevent coliform diarrhea in weaned pigs. Animal. (2020) 14:95–107. 10.1017/S1751731119002143 [DOI] [PubMed] [Google Scholar]

5.2.5 Ref. 13: Yap KM, Sekar M, Seow LJ, Gan SH, Bonam SR, Mat Rani NNI, et al. Mangifera indica (Mango): a promising medicinal plant for breast cancer therapy and understanding its potential mechanisms of action. Breast Cancer. (2021) 13:471–503. 10.2147/BCTT.S316667 [DOI] [PMC free article] [PubMed] [Google Scholar]

5.2.6 Ref. 14: Moga MA, Dimienescu OG, Balan A, Dima L, Toma SI, Bîgiu NF, et al. Pharmacological and therapeutic properties of Punica granatum phytochemicals: possible roles in breast cancer. Molecules. (2021) 26:1054. 10.3390/molecules26041054 [DOI] [PMC free article] [PubMed] [Google Scholar]

5.2.7 Ref. 15: Marcińczyk N, Gromotowicz-Popławska A, Tomczyk M, Chabielska E. Tannins as hemostasis modulators. Front Pharmacol. (2022) 12:806891. 10.3389/fphar.2021.806891 [DOI] [PMC free article] [PubMed] [Google Scholar]

5.2.8 Ref. 16: Gasaly N, Gotteland M. Interference of dietary polyphenols with potentially toxic amino acidmetabolites derived from the colonic microbiota. Amino Acids. (2021) 54:311–24. 10.1007/s00726-021-03034-3 [DOI] [PubMed] [Google Scholar]

5.2.9 Ref. 4: Maugeri A, Lombardo GE, Cirmi S, Süntar I, Barreca D, Laganà G, et al. Pharmacology and toxicology of tannins. Arch Toxicol.

(2022) 96:1257–77. 10.1007/s00204-022-03250-0 [DOI] [PubMed] [Google Scholar]

5.3 Additionally, "Gallotannins were proved to be potent inhibitors and disruptors of a series of bacteria (19). As documented, the outer membrane conferred bacterial resistance to microbicide (60). Benefiting from the strong affinity for iron, GTs showed inhibitory activities against the growth of bacteria. In response to the shin-whitening effect, GTs isolated from Chinese galls showed inhibitory activity against tyrosinase and inhibited the biosynthesis of melanin, which is related to hyperpigmentation (61). In a study on the antitumor mechanism, cytotoxicity against HepG2 and Chang hepatocellular carcinoma cells was exhibited by GTs (21). By inhibiting cell adhesion and suppressing cell repair motility, GTs would induce apoptosis of hepatocellular carcinoma cells."

5.3.1 Ref.19: Hricovíniová Z, Mascaretti Š, Hricovíniová J, Čížek A, Jampílek J. New unnatural gallotannins: a way toward greenantioxidants, antimicrobials and antibiofilm agents. Antioxidants. (2021) 10:1288. 10.3390/antiox10081288 [DOI] [PMC free article] [PubMed] [Google Scholar]

5.3.2. Ref. 60: Engels C, Schieber A, Ganzle MG. Inhibitory spectra and modes of antimicrobial action of gallotannins from mango Kernels (Mangifera indica L.). Appl Environ Microb. (2011) 77:2215–23. 10.1128/AEM.02521-10 [DOI] [PMC free article] [PubMed] [Google Scholar]

5.3.3. Ref. 61: Chen L-G, Chang W-L, Lee C-J, Lee LT, Shih CM, Wang CC. Melanogenesis inhibition by gallotannins from Chinese galls in B16 mouse melanoma cells. Biol Pharm Bull. (2009) 32:1447–52. 10.1248/bpb.32.1447 [DOI] [PubMed] [Google Scholar]

5.3.4. Ref. 21: Niemeta R, Gross GG. Enzymology of gallotannin and ellagitannin biosynthesis. Phytochem. (2005) 66:2001–11. 10.1016/j.phytochem.2005.01.009 [DOI] [PubMed] [Google Scholar]

5.4 Gallotannins are polymers formed when gallic acid, a polyphenol monomer, esterifies and binds with the hydroxyl group of a polyol carbohydrate such as glucose (1). They are characterized by a glycosidic core and galloyl unit, and are considered vital components of hydrolyzable tannins (3). Gallotannins are promptly hydrolyzed into gallic acid and glucose, making them an important dietary source of precursors for antioxidant compounds (5).”

5.4.1. ADD Ref. (1)

5.4.2. Add Ref. (3

5.4.3. Add Ref. (5)

6.0 **Which leads us to a consideration of Gallic Acid.**

6.1 “Gallic acid (also known as 3,4,5-trihydroxybenzoic acid) is a trihydroxybenzoic acid with the formula C6H2(OH)3CO2H. It is classified as a phenolic acid. It is found in gallnuts, sumac, witch hazel, tea leaves, oak bark, and other plants.[1] It is a white solid, although samples are typically brown owing to partial oxidation. Salts and esters of gallic acid are termed "gallates".”

6.1.1 “Gallic acid is easily freed from gallotannins by acidic or alkaline hydrolysis.”

6.1.2 “Hydrolyzable tannins break down on hydrolysis to give gallic acid and glucose or ellagic acid and glucose, known as gallotannins and ellagitannins, respectively.” (Andrew Pengelly (2004), The Constituents of Medicinal Plants (2nd ed.), Allen & Unwin, pp. 29–30)

6.2 “Carob fruit is a rich source of gallic acid (24–165 mg per 100 g).” (Goulas, Vlasios; Stylos, Evgenios; Chatziathanasiadou, Maria; Mavromoustakos, Thomas; Tzakos, Andreas (10 November 2016). "Functional Components of Carob Fruit: Linking the Chemical and Biological Space". International Journal of Molecular Sciences. 17 (11): 1875. doi:10.3390/ijms17111875. ISSN 1422-0067. PMC 5133875. PMID 27834921.)

6.3 “After consumption, about 70% of gallic acid is adsorbed and then excreted in the urine as 4-O-methylgallic acid. Differently, the ester derivatives of gallic acid, such as catechin gallate ester or gallotannins, are hydrolyzed to gallic acid before being metabolized to methylated derivatives. Gallic acid is a well known antioxidant compounds which has neuroprotective actions in different models of neurodegeneration, neurotoxicity and oxidative stress. In this review, we discuss about the neuroprotective actions of gallic acid and derivatives and their potential mechanisms of action." Polyphenols: well beyond the antioxidant capacity: gallic acid and related compounds as neuroprotective agents: you are what you eat!, Maria Daglia,, et.al., Curr Pharm Biotechnol. 2014;15(4):362-72. doi: 10.2174/1389201015041408251207 37.

6.4 Chios Mastic Gum is a resin derived from Pistacia lentiscus var. Chia and contains triterpenes (1,2,4,5). These triterpenes contribute to the biological and pharmacological properties of mastic, including its anti-inflammatory activity (1,2,4,5)

6.4.1 Ref.1: Triterpenoids from Chios mastic gum of Pistacia lentiscus and their inhibition of LPS-induced NO production in RAW 264.7 cells, Wei Liu, et.al., Tetrahedron, Volume 125, 22 October 2022, 133042, https://doi.org/10.1016/j.tet.2022.133042

6.4.2 Ref. 2: Anti-Inflammatory Activity of the Major Triterpenic Acids of Chios Mastic Gum and Their Semi-Synthetic Analogues, Panagiota Stamou, et.al., Biomolecules. 2024 Dec 18;14(12):1618. doi: 10.3390/biom14121618, PMCID: PMC11727566 PMID: 39766325

6.4.3 Ref. 4: Traditional uses, phytochemistry and pharmacology of Chios mastic gum (Pistacia lentiscus var. Chia, Anacardiaceae): A review, Vasiliki K. Pachi, et.al., Journal of Ethnopharmacology, Volume 254, 23 May 2020, 112485, https://doi.org/10.1016/j.jep.2019.112485

6.4.4 Ref. 5: Anti-inflammatory activity of Chios mastic gum is associated with inhibition of TNF-alpha induced oxidative stress, Angelike Triantafyllou, et.al., Nutr J. 2011 Jun 6;10:64. doi: 10.1186/1475-2891-10-64, PMCID: PMC3127998 PMID: 21645369

6.5 Triterpenes from Tabebuia avellanedae and Schinus terebinthifolius positively affect the cicatrization process in cattle skin wounds by promoting fibroplasty and wound decontamination. (1)

6.5.1 Ref. 1: Effects of 3 Topical Plant Extracts on Wound Healing in Beef Cattle, Leandro Cavalcante Lipinski, et.al., Afr J Tradit Complement Altern Med. 2012 Jul 1;9(4):542–547. doi: 10.4314/ajtcam.v9i4.11, PMCID: PMC3746646 PMID: 23983390

7.0 <u>One might ask: Is there an interrelation between polyphenol oxidase, gallotannins, and gallic acid (which are found in mastic and carob as well as being produced by Sugarcane Bagasse)?</u>

7.1 Here is what the science is suggesting to us:

7.1.1 Polyphenol Oxidases are regulated by Gallic Acid.

7.1.2 Gallotannins are produced from Gallic Acid and can revert back into Gallic Acid with ease.

7.2 We begin with polyphenol/polyphenol oxidases: "There is an interrelationship between polyphenol oxidases, Gallotannins, and Gallic Acid (1,2,3,4,5). Polyphenols influence LOX enzyme activity and expression levels, impacting the cross-linking (1). Polyphenols also have antioxidant properties and can increase endogenous antioxidant-synthesizing enzymes (3). In plants, the positive correlation between levels of PPO and resistance to pathogens and herbivores is observed (5)."

7.2.1 Ref.1: Exploring the Interplay between Polyphenols and Lysyl Oxidase Enzymes for Maintaining Extracellular Matrix Homeostasis, Carolina Añazco, et.al., Int J Mol Sci. 2023 Jul 1;24(13):10985. doi: 10.3390/ijms241310985

7.2.2 Ref. 2: Polyphenols—Gut Microbiota Interrelationship: A Transition to a New Generation of Prebiotics, Diana Plamada, et.al., Nutrients. 2021 Dec 28;14(1):137. doi: 10.3390/nu14010137

7.2.3 Ref. 3: How Do Polyphenol-Rich Foods Prevent Oxidative Stress and Maintain Gut Health?, Samir Jawhara, Microorganisms. 2024 Jul 31;12(8):1570. doi: 10.3390/microorganisms12081570

7.2.4 Ref. 4: Antioxidant and Pro-Oxidant Effects of Polyphenolic Compounds and Structure-Activity Relationship Evidence, Estela Guardado Yordi, et.al., Nutrition, Well-Being and Health, Chapter 2, February 2012, DOI:10.5772/29471

7.2.5 Polyphenol oxidases in plants and fungi: Going places? A review, Alfred M. Mayer, Phytochemistry, Volume 67, Issue 21, November 2006, Pages 2318-2331

7.3 "Polyphenol oxidase, also known as catechol oxidase or tyrosinase, is an enzyme that oxidizes phenolic compounds, causing enzymatic browning in fruits and vegetables (1). The rate of formation of colored benzoquinone is measured to assess polyphenol oxidase activity (2)."

7.3.1 Ref. 1: Flavonoid oxidation in plants: from biochemical properties to physiological functions, Lucille Pourcel, et.al., Trends in Plant Science, Volume 12, Issue 1, 2007, Pages 29-36, ISSN 1360-1385, https://doi.org/10.1016/j.tplants.2006.11.006.

7.3.2 Ref. 2: Plant defence mechanisms and enzymatic transformation products and their potential applications in food preservation: Advantages and limitations, Alma Fernanda Sanchez Maldonado, et.al., Trends in Food Science & Technology, Volume 46, Issue 1, November 2015, Pages 49-59.

7.3.2.1 A deeper dive into the study in Ref. 2 suggests, "Phenolic compounds are oxidised by polyphenol oxidase, also called catechol oxidase or tyrosinase, an enzyme that causes enzymatic browning in fruits and vegetables (Yoruk & Marshall, 2006). Polyphenol oxidases are type 3-copper containing oxidases, present in monomeric, dimeric and tetrameric forms (Dicko, Gruppen, Traoré, Voragen, & van Berkel, 2006). Polyphenol oxidases exist in the thylakoids of chloroplasts, vesicles or other compartments in non-green plastids (Vaughn, Lax, & Duke, 1988). They remain latent until they are released by physical tissue damage (Thipyapong et al., 2004). Then, they catalyse enzymatic browning to produce quinones, followed by non-enzymatic polymerization to generate melanin pigments (Fig. 2a)."

7.3.2.2 Of note here is the "physical tissue damage" that occurs in the milling of Sugarcane transforming in Sugarcane Bagasse (and the darkening with age), coupled with the formulation of Sugarcane Bagasse with Mastic, Carob, and Ionic Minerals processed in a manner that further shears the fiber; i.e., caused further tissue damage followed by accelerated "browning" of the transformed fiber.

7.3.2.3 The Ref. 2 study also tells us in the "Abstract: Phytoalexins and phytoanticipins are plant defence compounds that generally have antimicrobial properties; they include phenolic compounds, glucosinolates, cyanogenic glycosides, oxylipins and alkaloids, among others."

7.3.2.4 It Ref. 2 study further suggests: "Antioxidant, anti-inflammatory, and anticancer properties: Phytoalexins, such as glyceollins, stilbenoids, and isoflavones, have been reported to have various health benefits (1,2,3,4). These benefits include antioxidant, anti-inflammatory, and anticancer properties (2,3,4).

7.3.2.4.1 Ref. 1: Soy-derived phytoalexins: mechanism of in vivo biological effectiveness in spite of their low bioavailability, Jisun Oh, et.al., Food Sci Biotechnol. 2018 Oct 25;28(1):1–6. doi: 10.1007/s10068-018-0498-7

7.3.2.4.2 Ref. 2: Stilbenoids: Chemistry, occurrence, bioavailability and health effects—a review, Won Young Oh, et.al., J. Food Bioact. 2021;13:20–31

7.3.2.4.3 Ref. 3: Bioactive compounds' importance in plant-based beverages: a review, Karine

Marafon, et.al., Current Opinion in Food Science, Volume 63, June 2025, 101304

7.3.2.4.4 Ref. 4: Phytoalexins: Defend systems of plants and Pharmacological Potential -A systematic review, Sivakumar Thirumal, et.al., March 2023International Journal of Engineering Technology and Management Sciences Volume: 7(2):319-326, DOI:10.46647/ijetms.2023.v07i02.039

7.3.3 Please note that, "Sugarcane contains phytoalexins, including piceatannol; Sugarcane contains phytoalexins, including piceatannol, which inhibits spore germination and germ tube growth of fungal pathogens (1). These compounds help in the natural defense of plants against pests and diseases (3)."

7.3.4 Ref. 1: Isolation and identification of piceatannol as a phytoalexin from sugarcane, Anita M. Brinker, et.al., Phytochemistry, Volume 30, Issue 10, 1991, Pages 3229-3232

7.3.5 Ref. 3: Phytochemical profile of sugarcane and its potential health aspects, Amandeep Singh, et.al., Pharmacogn Rev. 2015 Jan-Jun;9(17):45–54. doi: 10.4103/0973-7847.156340

7.3.5.1 This Ref. 3 study states, "Sugarcane juice is widely used in India in the treatment of jaundice, hemorrhage, dysuria, anuria, and other urinary diseases. Herein, we have summarized the different phytoconstituents and health benefits of sugarcane and its valuable products. The phytochemistry of sugarcane wax (obtained from the leaves and stalks of sugarcane), leaves, juice, and its products has revealed the presence of various fatty acid, alcohol, phytosterols, higher terpenoids, flavonoids, -O- and -C-glycosides, and phenolic acids."

7.3.5.2 Furthermore, Phytoalexins are used to stimulate growth rate and increase weight gain in beef cattle (4). These growth promoters enhance muscle leanness, improve average daily gain, and enhance feed efficiency (1,2,3).

7.3.5.2.1 Ref. 1: AMERICAN MEAT ASSOCIATION REFERENCE PAPER SEPTEMBER 2014 Application of Growth Enhancing Compounds in Modern Beef Production, Executive Summary Bradley Johnson, Ph.D. and Jon Beckett, Ph.D.

7.3.5.2.2 Ref. 2: "Types and Uses of Growth Promoters," Mebrate Getabalew, et.al., Journal of Veterinary Medicine and Animal Sciences, Jul 17, 2020, Publisher: MedDocs Publishers LLC, Online edition: http://meddocsonline.org/

7.3.5.2.3 Ref. 3: PERFORMANCE AND GROWTH TECHNOLOGIES: BALANCING, BEEF QUALITY & YIELD

7.3.5.2.4 Ref. 4: Phytoestrogens: A Review of Their Impacts on Reproductive Physiology and Other Effects upon Grazing Livestock, Jessica Wyse, et.al., Animals (Basel). 2022 Oct 9;12(19):2709. doi: 10.3390/ani12192709

WHAT IS THE LINK

7.3.6 Of interests it the G-Unit of lignin: "Coniferyl alcohol is produced from coniferyl aldehyde by the action of dehydrogenase enzymes (1). It is one of the three building blocks found in lignin (2)." ?????

7.3.7 Lignans are phenylpropanoid dimmers synthesized via the monolignol pathway, with coniferyl alcohol as the direct precursor. They have vital roles in defense responses and can enhance plant resilience under stress and human health (4). ?????

7.3.8 Phytoestrogens

7.3.9 It is noted that “Lignin does not directly enhance phytoalexin production: The degradation of lignin does not directly enhance phytoalexin production (1,2,3,4,5). Phytoalexins, such as scopoletin and monolignols, are derived from phenylalanine and play a role in plant defense against pathogens (5). Lignin degradation is a complex process with various enzymes and mechanisms (2,3). While lignin degradation may indirectly affect plant defense, it is not a direct cause of increased phytoalexin."

7.3.9.1 Ref. 1:

7.3.9.2 Ref. 2:

7.3.9.3 Ref. 3:

7.3.9.4 Ref. 4:

7.3.9.5 Ref. 5:

7.3.10 HOWEVER, “Phenylalanine is naturally produced from degraded lignin: Phenylalanine is naturally produced from degraded lignin (1,2,3,4,5). Lignin is one of the most important secondary metabolites produced by the phenylalanine/tyrosine metabolic pathway in plant cells (1). Monolignols, including coniferol, are also produced from phenylalanine via a multistep phenylpropanoid pathway (2). Approximately 20-30% of photosynthetically fixed carbon is directed towards the synthesis of phenylalanine, which in turn contributes to lignin, the central structural component of plant cell walls3. Phenylalanine ammonia-lyase (PAL), the enzyme that converts phenylalanine to trans-cinnamic acid, is homologous to histidine ammonia-lyase (HAL), an enzyme involved in histidine degradation in primary metabolism4. The study of this metabolic pathway is particularly relevant in trees, which divert large amounts of carbon into the biosynthesis of phenyl-derived compounds, particularly lignin, an important constituent of wood (5).

7.3.10.1 Ref. 1:

7.3.10.2 Ref. 2:

7.3.10.3 Ref. 3:

7.3.10.4 Ref. 4:

7.3.10.5 Ref. 5:

7.3.11 And, the browning effect of Sugarcane Bagasse over time is like that of fruit, especially as the ligin is degraded and subsequently depolymerized. ADD Supporting Documentation

7.3.12 Furthermore, "There is a link between polyphenol oxidases (PPO) and gallic acid (1,3,4,5). Gallic acid is presented as a substrate and/or inhibitor of PPO (1). Gallic acid is a well-known antioxidant compound with neuroprotective actions (5).

7.3.12.1 Ref.1: Investigation of Kinetics of Polyphenol Oxidase-Gallic Acid Reaction in Selected Fruits, Sibel Yiğitarslan, et.al., IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB) ISSN: 2455-264X, Volume 6, Issue 1 (Jan. – Feb. 2020), PP 26-34 www.iosrjournals.org

7.3.12.1.1 Abstract: Enzymatic browning that causes losses in fruits occurs when the enzyme polyphenol oxidase (PPO) (monophenol oxidase E.C.1.14.18.1 or catechol oxidase E.C.1.10.3.1) reacts with oxygen. In this study, the interaction of PPO in red apple, banana and jerusalem artichoke with an antioxidant namely gallic acid was investigated. Gallic acid is presented in well-known database (BRENDA) as a substrate and/or inhibitor of the PPO. In order to investigate the reactions in between different concentrations of gallic acid solution (0-800 µl) with the PPO prepared, no additional substrate was added in its natural environment, and the media were analyzed by two methods. The findings of the study in a holistic way showed that the spectrophotometric and colorimetric method results of experiments of all fruits and all gallic acid concentrations studied were compatible with each other. It was observed that 50 µl stock gallic acid solution could stop the browning reaction, but when this concentration was exceeded, a different reaction which forms a green product was observed. In the study, the possible reaction mechanism between gallic acid-PPO was also determined. It was concluded that specific concentration of gallic acid can be used to inhibit PPO in production of goods, instead of using protective additives.

7.3.12.2 Ref. 3:

7.3.12.3 Ref. 4:

7.3.12.4 Ref. 5:

7.3.13 Furthermore, the browning effect reported to be caused by increased melanin, has also been shown per the study, "Association of pro-melanin concentrating hormone genotype with beef carcass quality and yield," L. J. Walter, et.al., Journal of Animal Science, Volume 92, Issue 1, January 2014, Pages 325–331, https://doi.org/10.2527/jas.2013-6931

7.3.13.1 In the "Abstract: Beef cattle from 3 independent studies conducted in the Texas Panhandle (Exp. 1: n = 3,906 and Exp. 2: n = 4,000) and southern Idaho (Exp. 3; n = 542) were used to investigate the association of pro-melanin concentrating hormone (PMCH) genotype with beef carcass quality and yield attributes."

7.3.13.1.1 The study continues with: "Pro-melanin concentrating hormone has been reported to play a role in the regulation of appetite and metabolism in mice and humans (Shimada et al., 1998; Elliott et al., 2004; Gavrila et al., 2005), and has been associated with beef tenderness (Helgeson, 2007). Helgeson and Schmutz (2008) mapped the pro-melanin concentrating hormone (PMCH) gene in Bos taurus cattle and discovered that cattle of the AA genotype had significantly more carcass subcutaneous fat than TT cattle in 2 separate populations (n = 122 and 382), indicating that PMCH genotype may influence carcass composition. In addition to an effect on carcass composition, PMCH genotype was associated with Warner–Bratzler shear force (WBSF) in longissimus dorsi steaks with AA genotypes exhibiting lower WBSF than AT and TT genotypes in steaks cooked to medium, whereas in steaks cooked to well done AA genotypes had a lower WBSF than AT cattle. Thus, based on previous literature, PMCH has been associated with beef carcass subcutaneous fat depth and tenderness."

7.3.13.1.2 The study continues to state that, "Further research to explore the association of PMCH and carcass composition could elucidate differences with respect to marbling score, yield grade, and HCW. This information could be used as a prediction tool for certain PMCH genotypes to be marketed sooner or marketed under different value-based grids to maximize producer financial returns. Furthermore, as tenderness is considered to be the single most important factor influencing consumers' perceptions of taste (Savell et al., 1987, 1989), specific PMCH genotypes may serve to derive a premium for the association with tenderness and subsequent consumer palatability. Multiple DNA tests (e.g., GeneSTAR® and HD 50K, Zoetis, Florham Park, NJ; Igenity®, Neogen, Lincoln, NE) have been developed linking DNA SNP to tenderness. However, few reports have investigated the PMCH alleles. Therefore, the objectives of this study were to assess the association of PMCH genotypes with attributes of carcass quality and yield and to confirm the association of PMCH genotypes with longissimus dorsi tenderness."

7.3.13.2 As a reminder, over a ½ dozen ranchers feeding SGP+™ as part of the Ration Management have harvested steers. All claim that meat quality and taste are beyond anything they have ever consumed. Furthermore, the butchers ask where they are getting beef of this quality.

7.3.13.3 Additionally, "Melanin affects the coat color of Angus cattle (1,2,3,4,5). The melanocortin 1 receptor (MC1R) gene controls the production of black and red pigments in the coats of domestic cattle5. The two main alleles for the MC1R gene are Black (ED) and Red (e)(2)."

7.3.13.3.1 Ref. 1:

7.3.13.3.2 Ref. 2:

7.3.13.3.3 Ref. 3:

7.3.13.3.4 Ref. 4:

7.3.13.3.5 Ref. 5:

ADD CONTENT

7.4 Polyphenol oxidase: Polyphenol oxidase (PPO) in bovine digestion reduces lipolysis and proteolysis in silo and in the rumen (3). PPO mediates protein-quinone binding, protecting plant proteins from proteolysis (1). Quinones formed by PPO indirectly affect the breakdown of lipases in the rumen (4). Laccases in the rumen may also play a role in lignin digestion (5).

7.4.1 Ref.1: "Increasing the rumen-stable protein content of feed would lead to improved nitrogen utilisation in cattle, and less nitrogenous waste. Red clover (Trifolium pratense L.) is a high protein ruminant feed containing high polyphenol oxidase (PPO) activity. PPO mediated protein-quinone binding has been linked to protecting plant proteins from proteolysis." (The effects of PPO activity on the proteome of ingested red clover and implications for improving the nutrition of grazing cattle, EH Hart, et.al., J Proteomics. 2016 Jun 1;141:67–76. doi: 10.1016/j.jprot.2016.04.023)

7.4.2 Ref.2: POLYPHENOL OXIDASE CONTAINING EXTRACT: SOURCES, Demissie CHANIE, et.al., and Ayalew H (2019). Online J. Anim. Feed Res., 9(4): 154-157. www.ojafr.ir

7.4.3 Ref. 3: Nitrogen and fatty acid rumen metabolism in cattle offered high or low polyphenol oxidase red clover silage, M R F Lee, et.al., Animal. 2018 Dec 19;13(8):1623–1634. doi: 10.1017/S1751731118003294

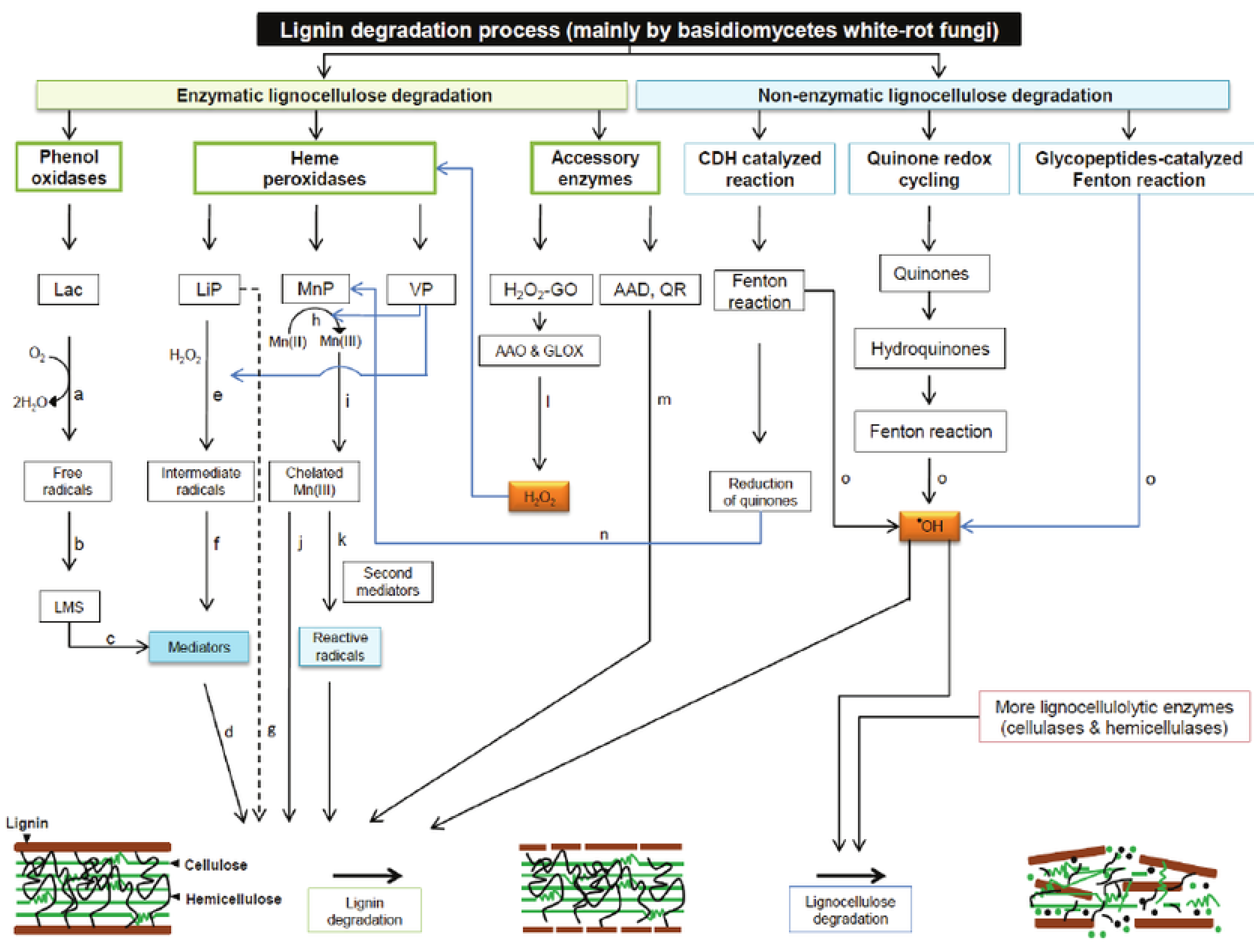
7.4.4 Ref. 4: Abstract: There is growing interest in specialized metabolites for fortification strategies in feed and/or as an antioxidant, anti-inflammatory and antimicrobial alternative for the containment of disorders/pathologies that can also badly impact human nutrition. In this context, the improvement 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. Often conflicting results emerge from a review of the literature of recent years. However, the data suggest pursuing a deepening of the role of phenols and polyphenols in ruminant feeding, paying greater attention to the chemistry of the single compound or to that of the mixture of compounds more commonly used for investigative purposes. Polyphenols for Livestock Feed: Sustainable Perspectives for Animal Husbandry?, Marialuisa Formato, et.al., Molecules. 2022 Nov 10;27(22):7752. doi: 10.3390/molecules27227752

7.4.5 Ref. 5: RL5, a gene coding for a novel polyphenol oxidase, was identified through activity screening of a metagenome expression library from bovine rumen microflora. Novel

Polyphenol Oxidase Mined from a Metagenome Expression Library of Bovine Rumen: BIOCHEMICAL PROPERTIES, STRUCTURAL ANALYSIS, AND PHYLOGENETIC RELATIONSHIPS, Ana Beloqui, et.al., Journal of Biological Chemistry, Volume 281, Issue 32, 11 August 2006, Pages 22933-22942

7.5 Let us now consider the degradation of lignin by White Rot Fungi. “Fungal biodegradation and enzymatic modification of lignin,” Mehdi Dashtban, et.al., Int J Biochem Mol Biol. 2010;1(1):36-50. Epub 2010 May 23.

Figure 1:



Lac: laccase, LMS: laccase-mediator system, LiP: lignin peroxidase, MnP: manganese peroxidase, VP: versatile peroxidase, H_2O_2-GO: H_2O_2-generating oxidases, AAO: aryl-alcohol oxidase, GLOX: glyoxal oxidase, H_2O_2: hydrogen peroxide AAD: aryl-alcohol dehydrogenases, QR: quinone reductases and $^{\bullet}OH$: free hydroxyl radicals.

7.3 Degradation of S-lignin-based derivatives

Sugarcane contains 60+% S-Lignin. “The S-lignin-based derivatives (syringic acid) contain two methoxy groups in its aromatic ring, which makes them more difficult to be degraded than G- and H-lignins. The S-lignin occupies 61.9%, 0, 58.9%, and 20.1% of the lignin in poplar, pine, corn, and Arabidopsis, respectively [110]. Compared with ferulic acid and p-coumaric acid, only a few microbes such as Sphingomonas sp. SYK-6 strain can metabolize syringic acid [120]. The demethylation of syringic acid is catalyzed to 3-O-methylgallate (3MGA) by tetrahydrofolate-dependent O-demethylase (DesA). The produced 3MGA can be converted to gallic acid (GA) and 4-carboxy-2-hydroxy-6-methoxy-6-oxohexa-2, 4-dienoate [120, 121], which are further transformed to 4-oxalomesaconate for acetyl-CoA synthesis. The intermediate 3MGA was then converted to 2-pyranone-4, 6-dicarboxylate, which is then converted to acetyl-CoA [122] (Fig. 6). Syringic acid was catalyzed to acetyl-CoA through multiple metabolic steps, which enters TCA cycle for cell growth and product synthesis. In addition to the typical lignin-derived aromatics, some aromatic compounds such as benzene, phenol, benzoate, toluene, and naphthalene can also be catalyzed to catechol [123].”

ADD CONTENT

8.0 **The aforementioned discussion leads us to the question: Do polyphenol oxidases, Gallotannins, and Gallic Acid effect mitochondrial function?**

8.1 “Currently, it is clear that part of the antioxidant properties of polyphenols might be due to their capacity to induced mitochondrial biogenesis and improve mitochondrial function, which increases the mitochondrial efficiency leading to reduction in ROS production.”

8.1.1 Effects of Polyphenols on Thermogenesis and Mitochondrial Biogenesis, Tanila Wood dos Santos, et.al., Int J Mol Sci. 2018 Sep 13;19(9):2757. doi: 10.3390/ijms19092757

9.0 **Gallic Acid and Mitochondria**

9.1 Is there fractal modification of mitochondria by gallic acid? “Gallic acid has been shown to modify mitochondria in several ways:

9.1.1 “It acts as a mitochondria-targeting vehicle with antioxidant properties, enhancing its therapeutic potential (1) (Gallic acid-mitochondria targeting sequence-H3R9 induces mitochondria-targeted cytoprotection, Yoonhee Bae, et.al., Korean J Physiol Pharmacol. 2022 Jan 1;26(1):15–24. doi: 10.4196/kjpp.2022.26.1.15)

9.1.2 “Gallic acid and rutin have been associated with increased mitochondrial DNA and enzymatic activities, indicating its beneficial effects on mitochondrial function (2). Rutin and Gallic Acid Regulates Mitochondrial Functions via the SIRT1 Pathway in C2C12 Myotubes, Wei-Tang Chang, et.al., Antioxidants (Basel). 2021 Feb 13;10(2):286. doi: 10.3390/antiox10020286.

9.1.3 “A new mitochondriotropic antioxidant based on gallic acid has been developed, which efficiently transports gallic acid to mitochondria without disrupting their function (3). Discovery of a new mitochondria permeability transition pore (mPTP) inhibitor based on gallic acid, José Teixeira, et.al., J Enzyme Inhib Med Chem. 2018 Mar 7;33(1):567–576. doi: 10.1080/14756366.2018.1442831.

9.1.4 “Gallic acid has protective effects against mitochondrial damage, particularly in response to harmful substances like bisphenol A (4). Gallic acid protects rat liver mitochondria ex vivo from bisphenol A induced oxidative stress mediated damages, Mousumi Dutta, et.al., Toxicol Rep. 2019 Jun 17;6:578–589. doi: 10.1016/j.toxrep.2019.06.011

9.1.5 “Additionally, gallic acid has been synthesized for its mitochondrial targeting, showing preventive effects against oxidative stress (5.) Efficiency of mitochondrially targeted gallic acid in reducing brain mitochondrial oxidative damage, P Parihar, et.al., Cell Mol Biol (Noisy-le-grand). 2014 Jul 3;60(2):35-41.”

9.2 "Gallic acid affects mitochondria by regulating oxidative stress, modulating mitochondrial dysfunction, and targeting mitochondria (1,2,3,4,5). It has antioxidative activity and can mitigate neuroinflammation (2, 5)."

9.2.1 Ref 1: Neuroprotective effects of gallic acid against hypoxia/reoxygenation-induced mitochondrial dysfunctions in vitro and cerebral ischemia/reperfusion injury in vivo, Jing Sun, et.al., Brain Research, Volume 1589, 17 November 2014, Pages 126-139

9.2.2 Ref 2: Gallic Acid and Gallates in Human Health and Disease: Do Mitochondria Hold the Key to Success?, Rekha Yamini Kosuru, et.al., Mol Nutr Food Res. 2018 Jan;62(1). doi: 10.1002/mnfr.201700699. Epub 2017 Dec 19.

9.2.3 Ref 3: Rutin and Gallic Acid Regulates Mitochondrial Functions via the SIRT1 Pathway in C2C12 Myotubes, Wei-Tang Chang, et.al., Antioxidants (Basel). 2021 Feb 13;10(2):286. doi: 10.3390/antiox10020286.

9.2.4 Ref 4: Gallic acid-mitochondria targeting sequence-H3R9 induces mitochondria-targeted cytoprotection, Yoonhee Bae, et.al., Korean J Physiol Pharmacol. 2022 Jan 1;26(1):15–24. doi: 10.4196/kjpp.2022.26.1.15

9.2.5 Ref. 5: Discovery of gallic acid-based mitochondriotropic antioxidant attenuates LPS-induced neuroinflammation, Shubham Garg, et.al., Free Radical Biology and Medicine, Volume 226, January 2025, Pages 302-329

9.3 Gallic acid, along with other mastic compounds, acts as a modulator of peroxisome proliferator-activated receptors (PPARs), which regulate glucose and lipid metabolism, inflammation, and fibrosis progression in the liver (2 & 5). (See Fig. 4 (Lower Right), Page79: "Summary of proposed intracellular catabolic pathways during lignocellulose degradation in WRF")

9.3.1 Ref. 2: "Overview of Chios Mastic Gum (Pistacia lentiscus) Effects on Human Health," Stergios Soulaidopoulos, et.al., rients. 2022 Jan 28;14(3):590. doi: 10.3390/nu14030590

9.3.2 Ref. 5: "Beneficial health effects of Chios Gum Mastic and peroxisome proliferator-activated receptors: indications of common mechanisms," Ioannis Georgiadis, et.al., J Med Food, 2015 Jan;18(1):1-10. doi: 10.1089/jmf.2014.0021.

9.3.2.1 "For thousands of years, Chios Gum Mastic (CGM), the resin produced by the trunk of Pistachia lentiscus var Chia, has been used for culinary and medicinal purposes and several therapeutic properties have been attributed to it. CGM has been used in traditional medicine of various nations in the eastern Mediterranean area. This survey was carried out to identify biological mechanisms that could explain traditional usage and recent pharmacological findings. We reviewed the related scientific literature available from the NCBI PUBMED database on CGM studies and on natural products showing peroxisome proliferator-activated receptor (PPAR) agonist effects. We investigated whether CGM qualifies as a PPAR modulator. A large number of studies demonstrate that CGM has antioxidant, anti-inflammatory, hypolipidemic, and anticancer properties. Recently, the first evidence of CGM antidiabetic effect became known. CGM chemical composition has been extensively analyzed and the presence of several compounds, especially triterpenoids is well documented. Some of them, oleanonic acid, oleanolic acid, and gallic acid are considered to act as PPAR modulators. PPARs are nuclear receptors functioning as transcription factors and thereby controlling cellular functions at the level of gene expression. PPARs are involved in the pathways of significant diseases, such as metabolic syndrome, diabetes mellitus, dyslipidemia, inflammation, atheromatosis, and neoplasias, constituting a key target for pharmacological interventions. This article proposes that the synergistic action of some constituents of CGM on PPARs and more precisely on both PPARs isotypes-α and -γ, may be one of the major biological mechanisms via which CGM exerts its multiple effects."

10.0 <u>Does this seemingly synergistic science, when overlayed, present evidence of the efficacy of SGP+™ on Bovine Herd Performance?</u>

10.1 "In this study, we showed that gallic acid was active against both Mannheimia haemolytica and Pasteurella multocida, two key BRD associated-pathogens, with the minimum inhibitory concentration (MIC) measured at 250 and 500 µg/mL, respectively." "Gallic Acid Potentiates the Antimicrobial Activity of Tulathromycin Against Two Key Bovine Respiratory Disease (BRD) Causing-Pathogens," Karthic Rajamanickam, et.al., Front Pharmacol. 2019 Jan 4;9:1486. doi: 10.3389/fphar.2018.01486.

10.2 "Bovine mastitis caused by *Staphylococcus aureus* may exacerbate by resulting in significant economic losses and impacting milk quality. To date, the use of gallic acid, a phenolic compound naturally occurring in various plants, holds promise due to its potent anti-oxidant and anti-inflammatory effects in many pieces of literature, thus, making it a subject of interest in bovine innate immunity research. Here we used gallic acid to assess its potential immunomodulation on milk phagocytes *in vitro* challenges with mastitis-causing bacteria. Our findings indicated that cells exposed to gallic acid showed no harm to cell viability but might maintain the longevity of cells during the bacterial infection. Gallic acid-treated cells displayed reduced cell migration, phagocytosis, and bacterial killing ability, while showing an increase in ROS production, all of which are undoubtedly linked to the intracellular killing abilities of the cells. Nonetheless, the extracellular structure called neutrophil extracellular traps (NETs) was significantly released after receiving gallic acid, representing extracellular killing. We also reported that gallic acid neutralizes inflammation by regulating specific pro-inflammatory genes (*IL1B*, *IL6*, *TNF*) and ROS-generating genes (*CYBA*, *LAMP1*, *RAC1*), subsequently preventing tissue damage. Regarding apoptosis-related genes and proteins, the increased production of caspase-3 and Bcl-2 family proteins could potentially promote the longevity of cells, implicated in the mechanism of combating bacterial invasion during udder inflammation and infection. The novel role of gallic acid on milk phagocytes highlights its potential immunomodulatory properties and contributes to our understanding of its effects on bacterial-host interactions, and provides valuable molecular insights." Gallic acid as a key substance to inhibit proliferation and adipogenesis in bovine subcutaneous adipocyte, Qing Jin, Anim Biotechnol. 2022 Aug;33(4):657-663. doi: 10.1080/10495398.2020.1822370. Epub 2020 Sep 18.

10.3 "Gallic acid (GA) has inhibitive effects on bovine subcutaneous adipocyte proliferation and adipogenesis. It activates the metabolic master factor AMP-activated protein kinase alpha (AMPKα) to promote programmed cell death and lipolysis. GA also reduces triglyceride levels and induces autophagy in bovine subcutaneous adipocytes." Gallic acid as a key substance to inhibit proliferation and adipogenesis in bovine subcutaneous adipocyte, Qing Jin, Anim Biotechnol. 2022 Aug;33(4):657-663. doi: 10.1080/10495398.2020.1822370. Epub 2020 Sep 18.

10.4 Gallic acid: a dietary metabolite's therapeutic potential in the management of atherosclerotic cardiovascular disease, Front. Pharmacol., Xiao-Lan Zhao, et.al., 06 January 2025, Cardiovascular and Smooth Muscle Pharmacology, Volume 15 – 2024, https://doi.org/10.3389/fphar.2024.1515172

10.5 Recently, we reported that GA exerts protective effects against inflammation. To test our hypothesis that the anti-inflammatory effect of GA partially contributes to the improvement of metabolic diseases, we examined the effect of GA on inflammation caused by adipocyte-macrophage crosstalk in obesity. We showed that GA enhanced adipocyte differentiation in 3 T3-L1 adipocytes. Consistent with the enhancement of adipogenesis, GA decreased the gene expression of monocyte chemoattractant protein-1 and increased that of adiponectin and the upstream mediator peroxisome proliferator-activated receptor gamma. GA also reduced inflammatory mediator expression induced by the co-culture of 3 T3-L1 adipocytes with RAW 264 macrophages.

10.5.1 Gallic acid regulates adipocyte hypertrophy and suppresses inflammatory gene expression induced by the paracrine interaction between adipocytes and macrophages in vitro and in vivo, Miori Tanaka, Nutr Res. 2020 Jan:73:58-66. doi: 10.1016/j.nutres.2019.09.007. Epub 2019 Oct 24.

10.5.2 Gallic acid has been studied for its effects on liver function in bovines. Some studies suggest that it may reduce liver steatosis, hepatic lipogenesis, and serum lipid levels.

However, more research is needed to confirm its effectiveness and mechanisms of action."

10.5.2.1 Oral gallic acid improve liver steatosis and metabolism modulating hepatic lipogenic markers in obese mice, Jaciara Neves Sousa, et.al., Experimental Gerontology, Volume 134, June 2020, 110881

10.5.3 Gallic acid reduces hepatic inflammation by modulating NF-κB pathway. Gallic acid boosts hepatic antioxidant enzymes in HFD-fed diabetic mice. Gallic acid inhibits NF-κB pathway, lowering lipid accumulation in steatohepatitis.

10.5.3.1 Gallic acid ameliorates diabetic steatohepatitis in db/db mice fed with a high-fat diet, Chi-Chih Wang, et.al., Journal of Agriculture and Food Research, Volume 19, March 2025, 101553

10.5.4 In conclusion, GA counteracted the progression of hepatic fibrosis through reduction of HSCs proliferation/activation mutually with their apoptosis induction.

10.5.4.1 Antifibrotic effects of gallic acid on hepatic stellate cells: In vitro and in vivo mechanistic study, Naglaa M El-Lakkany, et.al., J Tradit Complement Med. 2018 Apr 27;9(1):45–53. doi: 10.1016/j.jtcme.2018.01.010

10.5.5 "Diseases of the liver produce clinical signs of depression, anorexia, icterus, and sometimes photosensitization. Chronic liver disease is often accompanied by weight loss. Abdominal ultrasonography enables documentation of liver enlargement (more common in acute disease) or atrophy (more common in chronic disease), and it provides guidance for liver biopsy as a diagnostic and prognostic test. Depending on the specific cause, treatment often includes the administration of antimicrobials (if an infectious process is suspected), anti-inflammatories, and intravenous fluids with glucose, as well as sedation if neurologic signs are present from hepatic encephalopathy."

10.5.5.1 Overview of Hepatic Disease in Large Animals, Jonathan H. Foreman, DVM, DACVIM, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Reviewed/Revised May 2023 | Modified Sept 2024

11.0 In yet another study (van Vliet, Stephan & Provenza, Frederick & Kronberg, Scott. (2021). "Health-Promoting Phytonutrients Are Higher in Grass-Fed Meat and Milk," Frontiers in Sustainable Food Systems. 4. 555426. 10.3389/fsufs.2020.555426.), "Emerging data indicate that when livestock are eating a diverse array of plants on pasture, additional health-promoting phytonutrients—terpenoids, phenols, carotenoids, and anti-oxidants—become concentrated in their meat and milk. Several phytochemicals found in grass-fed meat and milk are in quantities comparable to those found in plant foods known to have anti-inflammatory, anti-carcinogenic, and cardioprotective effects. As meat and milk are often not considered as sources of phytochemicals, their presence has remained largely underappreciated in discussions of nutritional differences between feedlot-fed (grain-fed) and pasture-finished (grass-fed) meat and dairy, which have predominantly centered around the ω-3 fatty acids and conjugated linoleic acid. Grazing livestock on plant-species diverse pastures concentrates a wider variety and higher amounts of phytochemicals in meat and milk compared to grazing monoculture pastures, while phytochemicals are further reduced or absent in meat and milk of grain-fed animals. The co-evolution of plants and herbivores has led to plants/crops being more productive when grazed in accordance with agroecological principles. The increased phytochemical richness of productive vegetation has potential to improve the health of animals and upscale these nutrients to also benefit human health. Several studies have found increased anti-oxidant activity in meat and milk of grass-fed vs. grain-fed animals."

12.0 POLYPHENOLS: Phenolic Acids: Hydroxybenzoic acid

12.1 In the study be Gasaly, Naschla & Gotteland, Martin in (2022), "Interference of dietary polyphenols with potentially toxic amino acid metabolites derived from the colonic microbiota," Amino Acids. 54. 3. 10.1007/s00726-021-03034-3, itr was demonstrated that "Domesticated ruminants do not express proline-rich proteins in their saliva (Austin et al. 1989) and it has been proposed that tannins could bind dietary plant proteins in the rumen (pH 5.5–7.0), preventing their microbial degrada tion by the commensal microbiota. In the abomasum where pH is lower (2.5–3.5), the non-covalent linkages between proteins and tannins are broken, releasing free proteins that can be absorbed in the distal small intestine (Bunglavan et al. 2013). Therefore tannin-bound proteins should be more resistant to bacterial degradation in the rumen, and tannin supplementation would improve protein utilization and nitrogen balance and decreases the emission of gas and other amino acid-derived microbial metabolites in ruminants (Jayanegara et al. 2012; Min et al. 2015). Though the pres ence of bacterial populations capable of degrading in vitro the tannin/protein complexes has been reported in wild and domesticated ruminants (Goel et al. 2005), their physiologi cal importance in situ remains unclear."

12.2 The study continues with, "The effect of tannin supplementation was also evaluated in ruminants. As described above, in these animals, dietary proteins are rapidly degraded by the rumen microbiota to peptides and amino acids that are used by these microorganisms as nitrogen and energy source. Accordingly, pro tein bioavailability in the small intestine decreases, while the production of ammonia and other metabolites such as indole and skatole is enhanced, deteriorating milk and meat quality, and increasing manure emissions. The daily administration of quebracho tannins (8.93% in food, 40.3 g of tannic acid equivalent per kg DM.) in male lambs for 60d did not affect indole levels in the rumen and fat tissue but decreased skatole concentrations and improved meat odor (Priolo et al. 2009)

12.3 The dietary supplementation (0, 4.5, 9, and 18 g/kg) of lactating Holstein dairy cows with a mixture of red quebracho (2/3) and chestnut (1/3) tannin extracts contain ing 79% tannins resulted in a decrease in ammonia emission by 16 and 8% in the animals fed a diet with low (155 g/kg) or high (168 g/kg) level of protein, respectively, compared with the controls (Powell et al. 2011). Fecal urease activity was attenuated in the supplemented animals and direct spread ing of tannins on barn floors also lowered NH_3 emissions. Similar results were reported after the direct application of different types of tannins to ready-to-spread farm-made manure slurry from cattle, which reduced pH and ammonia emissions by more than 95% (Sepperer et al. 2020).

12.4 Tannin impact has also been evaluated in vitro. The addi tion of PACs from the forage legume, Dorycnium rectum, to rumen inocula reduced by 75% the formation of skatole and indole, and specifically inhibited by 85% the transforma tion of indoleacetic acid to skatole (Tavendale et al. 2005). Similar diminutions of skatole, indole, and ammonia were reported by Schreurs et al. in in vitro rumen fermentation of fresh white clovers (which does not contain PACs) alone or with growing concentrations of the PAC-containing for age Lotus pedunculatus, or PACs extracted from this forage (Schreurs et al. 2007). Such decrease was inhibited in pres ence of polyethylene glycol, a compound that binds PACs with high affinity, preventing them to bind proteins. The addition of tryptophan to the incubation medium increased indole and skatole production, indicating that the bacteria implicated in their formation were not affected by the PACs.

13.0 <u>The Topic: Phenolic Acids: Hydroxybenzoic acids: 4-Hydroxybenzoic acid contained and/or produced by Carob, Chios Mastic Gum, and Depolymerized Lignin from Sugarcane Bagasse.</u> (Of Note: 4-Hydroxybenzoic acid is isomeric with 2-hydroxybenzoic acid, known as salicylic acid, a precursor to aspirin, and with 3-hydroxybenzoic acid.)

13.1 This is but one more ingredient, which when considered in the context of the biochemical processes of the metabolic pathways associated with mammalian physiology, presents to us another group of plausible explanations as to the efficacy of Nutri-Mastic™, SGP+™, and SupremeAG™.

13.1.1 Like all things, too much of anything is a problem. Conversely, not enough to that thing is also a problem. Hence, striking a balance so as to create and maintain a level of healthy stasis in a multi-dimensional dynamic environment is both the challenge and the task at hand. (Remember the Map of Metabolic Pathways in IFUS Part 1 White Paper.) Such is the case with 4-Hydroxybenzoic acid.

13.1.2 Hence, we consider another thread seemingly (1) impacting gut microbiota, (2) their respective individual and collective functions, (3) the health benefits (if these functions are in statis) and (4) health consequences (if these functions are not).

13.1.3 As a reminder, it is the Health and Wellness of the person, animal, and/or the plant that is the absolute Performance Measure. All other things are simply indicators and a guidance system to provides data, information, knowledge, and/or actual intelligence (NOT AI; i.e., Artificial Ignorance).

13.1.4

13.2 All the ingredients in both Nutri-Mastic™, SGP+™, and SupremeAG™ contain 4-Hydroxybenzoic acid:

13.2.1 "4-Hydroxybenzoic acid can be found naturally in coconut.[3] It is one of the main catechins metabolites found in humans after consumption of green tea infusions.[4] It is also found in wine,[5] in vanilla, in Macrotyloma uniflorum (horse gram), carob [6] and in Phyllanthus acidus (Otaheite gooseberry)."

13.2.1.1 Ref.(6) Components of Carob Fruit: Linking the Chemical and Biological Space". International Journal of Molecular Sciences. 17 (11): 1875. doi:10.3390/ijms17111875. PMC 5133875. PMID 27834921.

13.2.2 Pistacia lentiscus (Chios Mastic Gum) contains 4-Hydroxybenzoic acid (1, 2, 5)

13.2.2.1 Ref.(1) Aghiles Karim Aissat, Nassima Chaher-Bazizi, Tristan Richard, Dina Kilani-Atmani, Eric Pedrot, Elodie Renouf, Djebbar Atmani, Josep Valls Fonayet, Analysis of individual anthocyanins, flavanols, flavonols and other polyphenols in Pistacia lentiscus L. fruits during ripening, Journal of Food Composition and Analysis, Volume 106,2022,104286, ISSN 0889-1575, https://doi.org/10.1016/j.jfca.2021.104286. (https://www.sciencedirect.com/science/article/pii/S0889157521004865)

13.2.2.2 Ref. (2) Ouahabi, S.; Loukili, E.H.; Elbouzidi, A.; Taibi, M.; Bouslamti, M.; Nafidi, H.-A.; Salamatullah, A.M.; Saidi, N.; Bellaouchi, R.; Addi, M.; et al. Pharmacological Properties of Chemically Characterized Extracts from Mastic Tree: In Vitro and In Silico Assays. Life 2023, 13, 1393. https://doi.org/10.3390/life13061393

13.2.2.3 Ref. (5) Phenolic Compounds Characterization from Pistacia lentiscus (lentisc) Fruit , Hajer Trabelsi et al, J. Chem. Pharm. Res., 2016, 8(8):1-8, ISSN : 0975-7384, CODEN(USA) : JCPRC5

13.2.3 Depolymerized lignin from sugarcane bagasse can produce 4-Hydroxybenzoic acid (1, 3)

13.2.3.1 Ref.(1) Saleh Al Arni, Extraction and isolation methods for lignin separation from sugarcane bagasse: A review, Industrial Crops and Products, Volume 115, 2018, Pages 330-339, ISSN 0926-6690, https://doi.org/10.1016/j.indcrop.2018.02.012.

13.2.3.2 Ref.(3) A Review: Depolymerization of Lignin to Generate High-Value Bio-Products: Opportunities, Challenges, and Prospects, Ningning Zhou, et.al., Front. Energy Res., 10 January 2022, Sec. Bioenergy and Biofuels, Volume 9 - 2021 | https://doi.org/10.3389/fenrg.2021.758744

13.3 The Good: 4-Hydroxybenzoic acid (4-HBA and also known as p-hydroxybenzoic acid (PHBA)) has several beneficial health effects, including anticancer, neuroprotective, and cardioprotective effects. It is also a precursor of coenzyme Q10 (CoQ10). Sources of 4-HBA include coconut, green tea, olive products, berries, wine, coriander, almonds, and other plants, with production also occurring through bacterial strains in the microbiome (1, 2)

13.3.1 Ref.(1) https://www.rupahealth.com/biomarkers/4-hydroxybenzoic-acid

13.3.2 Ref. (2) Chaudhary, Jasmine, et.al., 2013/09/01, A Comprehensive Review on Biological activities of p-hydroxy benzoic acid and its derivatives, Vol. 2, International Journal of Pharmaceutical Sciences Review and Research

13.4 The Good 1: For Humans, Nutri-Mastic™ (specifically the Mastic) contains 4-Hydroxybenzoic Acid. In a study conducted by Herebian D, Seibt A, Smits SHJ, Rodenburg RJ, Mayatepek E, Distelmaier F. 4-Hydroxybenzoic acid restores CoQ10 biosynthesis in human COQ2 deficiency. Ann Clin Transl Neurol. 2017 Oct 17;4(12):902-908. doi: 10.1002/acn3.486. PMID: 29296619; PMCID: PMC5740244.

13.4.1 "The clinical phenotypes of human CoQ10-deficiency caused by COQ2 mutations range from fatal neonatal disease to adult-onset multisystem atrophy. So far, treatment options for these diseases are unsatisfactory. Here, we demonstrate that supplementation of 4-hydroxybenzoic acid (4-HBA) fully restores endogenous CoQ10-biosynthesis in COQ2-deficient cell lines. This was accompanied by increased protein expression of CoQ10-biosynthesis-enzymes as well as a rescue of cell viability during stress conditions. In silico analysis suggested a ligand transportation path for 4-HBA through the COQ2 protein towards the mitochondrial matrix side. This process is apparently hindered by disease-causing mutations, which can be overcome by increasing 4-HBA concentrations."

13.5 The Good 2: For Humans, Nutri-Mastic™ (specifically the Mastic) contains 4-Hydroxybenzoic Acid . In a study "4-Hydroxybenzoic acid for multiple system atrophy?, Parkinsonism & Related Disorders," Volume 50, 2018, Pages 119-120, ISSN 1353-8020, https://doi.org/10.1016/j.parkreldis.2018.01.019. (https://www.sciencedirect.com/science/article/pii/S1353802018300348)

13.5.1 Abstract: Increasing evidence supports a link between multiple system atrophy and coenzyme Q10 (CoQ10) biosynthesis. However, so far this knowledge was not translated into tangible benefits for affected patients. Poor bioavailability of oral CoQ10 might constitute a major problem. Current research suggests that 4-hydroxybenzoic acid might constitute an interesting alternative treatment option.

13.6 The Good 3: For both humans and animals another study shows, "4-Hydroxybenzoic acid has several benefits for animals per a study, 4-Hydroxybenzoic acid rescues multisystemic disease and perinatal lethality in a mouse model of mitochondrial disease," (Julia Corral-Sarasa, et.al., Cell Reports, Volume 43, Issue 5114148, May 28, 2024, https://doi.org/10.1016/j.celrep.2024.114148)

13.6.1 The Good 4: For both humans and animals further study shows, "A Comprehensive Review on Biological activities of p-hydroxy benzoic acid and its derivatives,"(Jasmine Chaudhary, et.al., September 2013International Journal of Pharmaceutical Sciences Review and Research 22(2)):

13.6.1.1 It rescues multisystemic disease and perinatal lethality in a mouse model of CoQ deficiency.

13.6.1.2 It stimulates endogenous CoQ biosynthesis in tissues of Coq2 mutant mice.

13.6.1.3 It shows antifungal, antimutagenic, antisickling, estrogenic, and antimicrobial activities.

13.6.1.4 It has a growth stimulation effect on freshwater green alga.

13.6.1.5 Some derivatives of 4-hydroxybenzoic acid inhibit acetic acid-induced edema and are used in the management of sickle cell disease.

13.6.1.6 The alkyl esters of para-hydroxybenzoic acid are commonly used to preserve cosmetics, toiletries, drugs, and food products

13.7 The Good 5: For Humans, "Increasing evidence supports a link between multiple system atrophy and coenzyme Q10 (CoQ10) biosynthesis. However, so far this knowledge was not translated into tangible benefits for affected patients. Poor bioavailability of oral CoQ10 might constitute a major problem. Current research suggests that 4-hydroxybenzoic acid might constitute an interesting alternative treatment option." Felix Distelmaier, 4-Hydroxybenzoic acid for multiple system atrophy?, Parkinsonism & Related Disorders, Volume 50, 2018, Pages 119-120, ISSN 1353-8020, https://doi.org/10.1016/j.parkreldis.2018.01.019. (https://www.sciencedirect.com/science/article/pii/S1353802018300348)

13.8 The Good 6: “4-Hydroxybenzoic acid (HBA) has been found to be neuroprotective against oxidative stress. It is a metabolite of pelargonidin-based anthocyanins and may possess significant antioxidant abilities in vitro. HBA supplementation has been shown to rescue multisystemic disease and perinatal lethality in a mouse model of CoQ deficiency.” (Hence, when IFUS reports impacts of customers taking Nutri-Mastic™ as it applies to Autism, ADHD, improved cognitive function, or other such improvements, here is plausible science that provides insight into “How and Why?”

13.8.1 Winter AN, Brenner MC, Punessen N, Snodgrass M, Byars C, Arora Y, Linseman DA. Comparison of the Neuroprotective and Anti-Inflammatory Effects of the Anthocyanin Metabolites, Protocatechuic Acid and 4-Hydroxybenzoic Acid. Oxid Med Cell Longev. 2017;2017:6297080. doi: 10.1155/2017/6297080. Epub 2017 Jun 27. PMID: 28740571; PMCID: PMC5504963.

13.8.2 Winter AN, Bickford PC. Anthocyanins and Their Metabolites as Therapeutic Agents for Neurodegenerative Disease. Antioxidants (Basel). 2019 Aug 22;8(9):333. doi: 10.3390/antiox8090333. PMID: 31443476; PMCID: PMC6770078.

13.9 The Not So Good…when in excess or not properly metabolized: 4-Hydroxybenzoic acid has estrogenic activity both in vitro and in vivo,[15] and stimulates the growth of human breast cancer cell lines.[16][17] It is a common metabolite of paraben esters, such as methylparaben.[15][16][17] The compound is a relatively weak estrogen, but can produce uterotrophy with sufficient doses to an equivalent extent relative to estradiol, which is unusual for a weakly estrogenic compound and indicates that it may be a full agonist of the estrogen receptor with relatively low binding affinity for the receptor.[16][18][19] It is about 0.2% to 1% as potent as an estrogen as estradiol.[18] (As a note, there is NO evidence to date to indicate that the application of Nutri-Mastic™, SGP+™, and SupremeAG™ have produced negative impacts when applied as recommended.

13.9.1 Ref. (15) https://hmdb.ca/metabolites/HMDB0000500

13.9.2 Ref. (16) Byford JR, Shaw LE, Drew MG, Pope GS, Sauer MJ, Darbre PD. Oestrogenic activity of parabens in MCF7 human breast cancer cells. J Steroid Biochem Mol Biol. 2002 Jan;80(1):49-60. doi: 10.1016/s0960-0760(01)00174-1. PMID: 11867263.

13.9.3 Ref. (17) Lemini C, Jaimez R, Avila ME, Franco Y, Larrea F, Lemus AE. In vivo and in vitro estrogen bioactivities of alkyl parabens. Toxicol Ind Health. 2003 Jul;19(2-6):69-79. doi: 10.1191/0748233703th177oa. PMID: 15697177.

13.9.4 Ref.(18) Jasmine Chaudhary, et.al., ,A Comprehensive Review on Biological activities of p-hydroxy benzoic acid and its derivatives, September 2013International Journal of Pharmaceutical Sciences Review and Research 22(2)

13.9.5 Ref.(19) Nguyen, Q.N.; Lee, S.R.; Kim, B.; Hong, J.-H.; Jang, Y.S.; Lee, D.E.; Pang, C.; Kang, K.S.; Kim, K.H. Estrogenic Activity of 4-Hydroxy-Benzoic Acid from Acer tegmentosum via Estrogen Receptor α-Dependent Signaling Pathways. Plants 2022, 11, 3387. https://doi.org/10.3390/plants11233387

13.10 Based on reports from Beef Ranchers and Dairymen applying SGP+™ to respective herds as part of their respective Ration Management Strategy, (1) milk bags, (2) colostrum, (3) infant mortality, (4) calf health and well-being, and (5) increased calving. This in turn begs the question once again as to "Why and How?"

13.10.1 The "Why and How? may be explained by the interplay of 4-Hydroxybenzoic acid and White Rot Fungi. As a reminder, "White-rot fungi (WRF) are efficient organisms for lignin degradation in nature. Recent studies have shown that WRF can utilize lignin-related aromatic compounds, such as 4-hydroxybenzoic acid (4HBA), as carbon sources."

13.10.1.1 In the study performed by Monteiro, L.M.O., del Cerro, C., Kijpornyongpan, T. et al. "Metabolic profiling of two white-rot fungi during 4-hydroxybenzoate conversion reveals biotechnologically relevant biosynthetic pathways," (Commun Biol 8, 224 (2025).

https://doi.org/10.1038/s42003-025-07640-9), it was shown that:

13.10.1.1.1 "White-rot fungi are efficient organisms for the mineralization of lignin and polysaccharides into CO2 and H2O. Despite their biotechnological potential, WRF metabolism remains underexplored. Building on recent findings regarding the utilization of lignin-related aromatic compounds as carbon sources by WRF, we aimed to gain further insights into these catabolic processes. For this purpose, Trametes versicolor and Gelatoporia subvermispora were incubated in varying conditions – in static and agitation modes and different antioxidant levels – during the conversion of 4-hydroxybenzoic acid (a lignin-related compound) and cellobiose. Their metabolic responses were assessed via transcriptomics, proteomics, lipidomics, metabolomics, and microscopy analyses. These analyses reveal the significant impact of cultivation conditions on sugar and aromatic catabolic pathways, as well as lipid composition of the fungal mycelia. Additionally, this study identifies biosynthetic pathways for the production of extracellular fatty acids and phenylpropanoids – both products with relevance in biotechnological applications – and provides insights into carbon fate in nature."

13.10.1.2 As an added note, 4-Hydroxybenzoic acid (4-HBA) can be naturally converted into 4-hydroxybenzoate (and vice versa) in the following ways: (1) In prokaryotes, 4-HBA forms from chorismate via the shikimate pathway and (2) In microbes, chorismate can be converted into 4-hydroxybenzoate and pyruvate by the chorismate-pyruvate-lyase enzyme. (https://www.chemicalbook.com/article/4-hydroxybenzoic-acid-synthesis-method-and-biological-activity.htm)

13.11 Hence, one might offer a plausible theory that the Mastic and the Carob added to Sugarcane Bagasse improves the efficacy of White Rot Fungi (and possibly other components in the biome of Sugarcane Bagasse) to both degrade and depolymerize the lignin; hence, putting into motion the interplay of 4-Hydroxybenzoic acid CoQ10-biosynthesis in COQ2-deficient cell lines.

13.12 Carob fruit contains benzoic acids, including syringic acid, 4-hydrobenzoic acid, and gentisic acid. (Goulas V, Stylos E, Chatziathanasiadou MV, Mavromoustakos T, Tzakos AG. Functional Components of Carob Fruit: Linking the Chemical and Biological Space. Int J Mol Sci. 2016 Nov 10;17(11):1875. doi: 10.3390/ijms17111875. PMID: 27834921; PMCID: PMC5133875.)

13.12.1 Pistacia lentiscus contains benzoic acid. (Floris S, Di Petrillo A, Pintus F, Delogu GL. Pistacia lentiscus: Phytochemistry and Antidiabetic Properties. Nutrients. 2024 May 27;16(11):1638. doi: 10.3390/nu16111638. PMID: 38892571; PMCID: PMC11174566.)

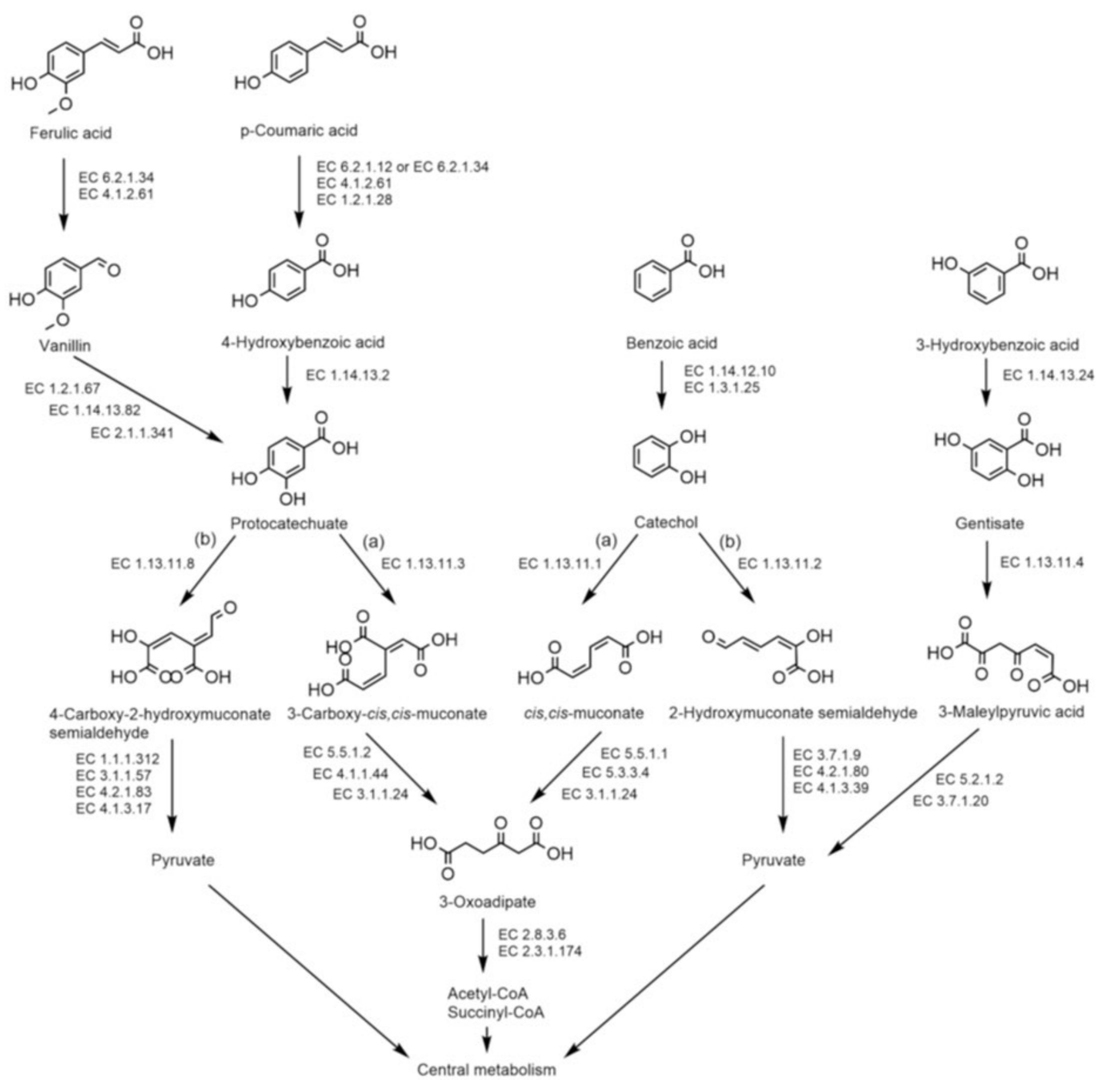
13.12.2 As a note, Benzoic acid was offered as a supplement to beef and day cattle. The following study suggests no improvements noted other than impacts to DMI and ruminal pH. As a rule, IFUS does not believe that individual supplements work per se as these must be introduced as part of integrated ration management systems.

13.12.2.1 Williams MS, Mandell IB, Bohrer BM, Wood KM. The effects of feeding benzoic acid and/or live active yeast (Saccharomyces cerevisiae) on beef cattle performance, feeding behavior, and carcass characteristics. Transl Anim Sci. 2021 Oct 1;5(4):txab143. doi: 10.1093/tas/txab143. PMID: 34877478; PMCID: PMC8643465.

13.12.2.1.1 "Implications: This preliminary research on benzoic acid in high-grain finishing diets may indicate potential as an antibiotic alternative for feedlot cattle. Results also show that the supplementation of benzoic acid, active live Saccharomyces cerevisiae, or in a combination in finisher diets with monensin did not impact carcass characteristics or performance, but BA supplementation increased DMI relative to control and yeast, suggesting no negative impacts on feed intake."

13.12.3 Furthermore, per the biochemical pathway illustrated below, the interplay between 4-Hydroxybenzoic acid, Benzoic Acid, and other compounds contained in the natural ingredients found in SGP+™ (e.g., Ferulic Acid, p-Coumaric Acid, Vanillin, etc.)

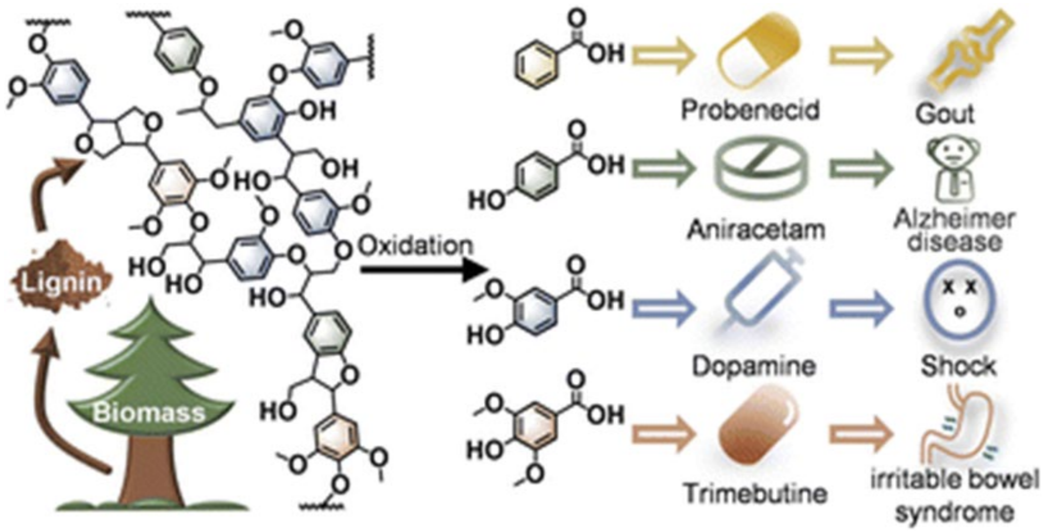
13.12.4



13.12.4.1 The diagram above is further supported by "Sustainable production of active pharmaceutical ingredients from lignin-based benzoic acid derivatives via "demand orientation"," Yuguo Dong, et.al., Green Chemistry, Issue 10, 2023 https://pubs.rsc.org/en/content/articlelanding/2023/gc/d3gc00241a

13.12.4.1.1 Abstract: Research related to lignin valorization into fine chemicals is an extremely demanding task due to the wide distribution and low economic value of depolymerization products. Benzoic acid derivatives (BADs) are the final products of the selective oxidation of the lignin side chain (i.e., p-hydroxybenzoic acid, vanillic acid, syringic acid, and benzoic acid), with favorable processibility functionality, showing great potential in the synthesis of active pharmaceutical ingredients (APIs) containing ester and carbonyl groups. Herein, this tutorial review presents our views on lignin utilization, especially using lignin-based benzoic acid derivatives (LBADs) as raw materials for the synthesis of APIs, with the aim of providing a greener, more eco-friendly approach for providing a sustainable route for the production of APIs. We first introduce the conversion of lignin or lignin platform compounds via the oxidative cleavage of C–C and C–O bonds to LBADs. Subsequently, focusing on the functional group modification strategy for the conversion of LBADs, we raise a "Demand Orientation" concept and several typical API synthesis routes. This tutorial review provides green production routes from LBADs in six instances (i.e., trimebutine, aniracetam, diethylstilbestrol, dopamine, acetaminophen, oxybuprocaine). Finally, several others APIs from LBADs via theoretically feasible and sustainable routes are proposed. Some personal perspectives are provided to highlight the opportunities within this attractive field.

13.12.4.1.2 Graphical abstract: Sustainable production of active pharmaceutical ingredients from lignin-based benzoic acid derivatives via "demand orientation"



13.12.4.2 Also, the diagram below illustrates a view of Central Metabolic Pathways driven in this view by Pyruvate (and as a continuation from the diagram in 11f:

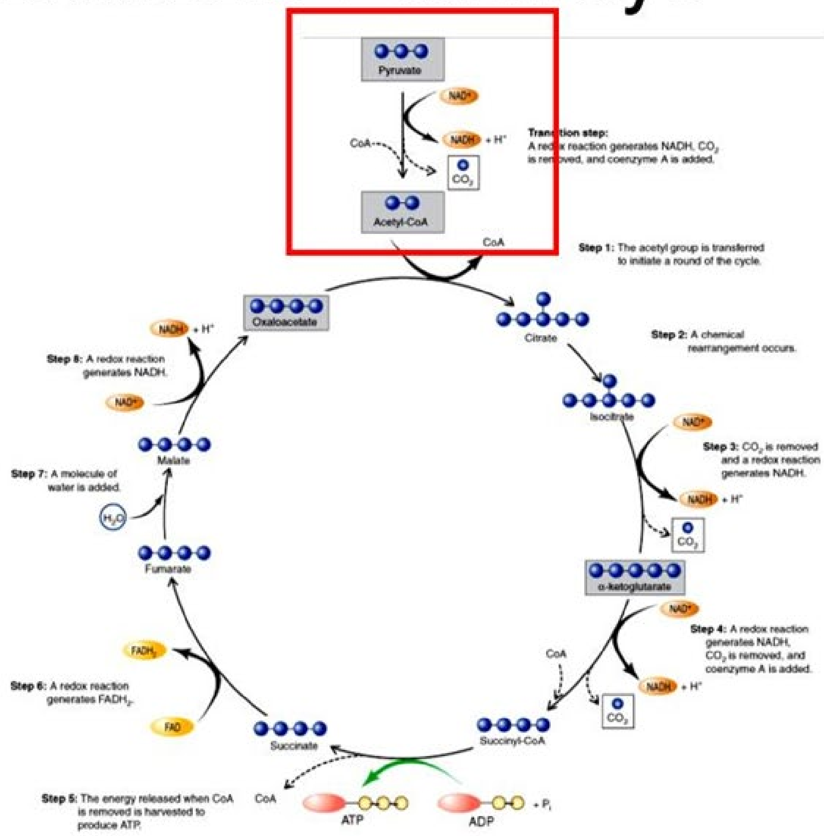
Central Metabolic Pathways

Transition step

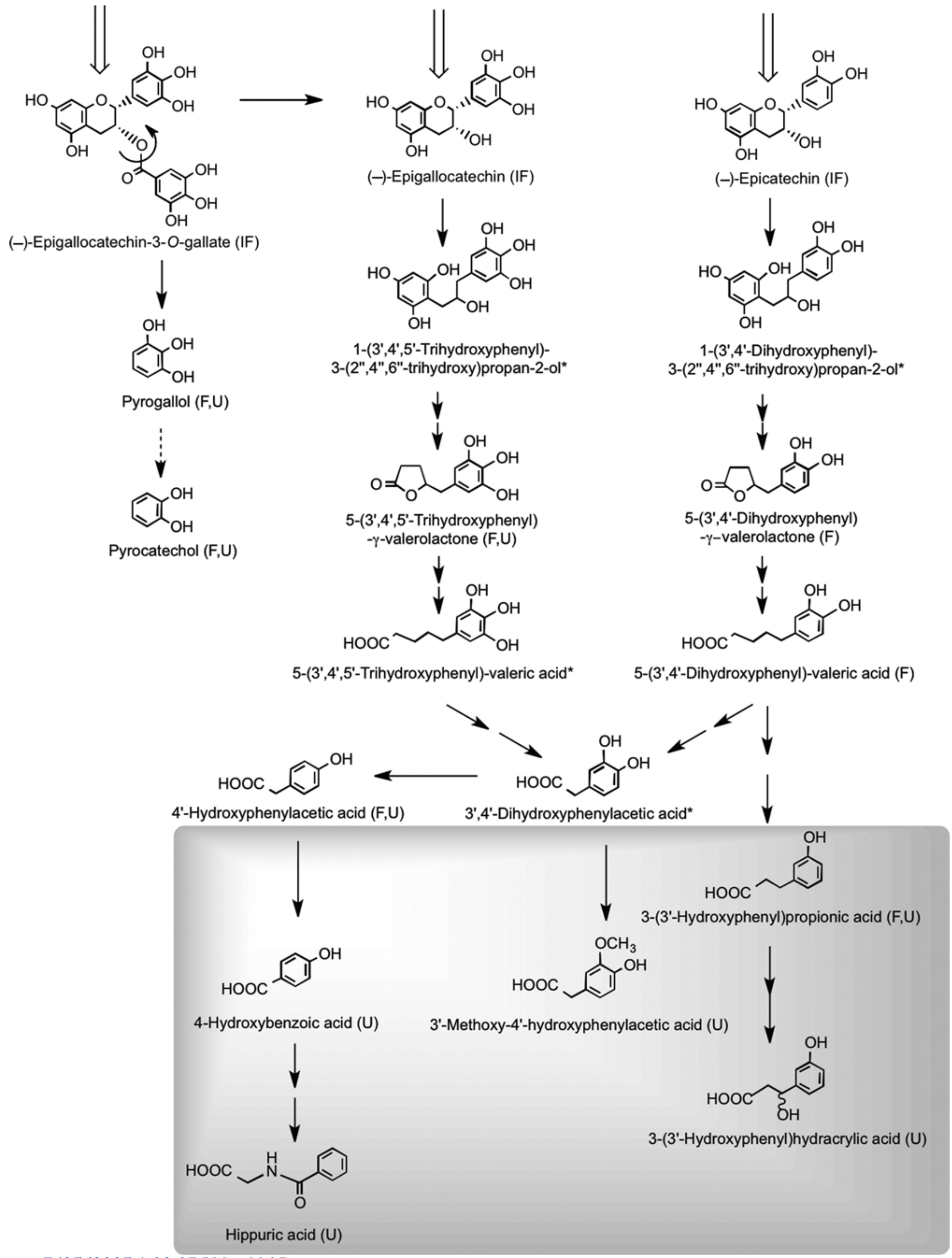
pyruvate (3 C) → acetyl CoA (2 C) + CO_2

(twice per glucose)

- •NADH
- •precursor metabolite



13.13 Another pathway involving 4-Hydroxybenzoic acid results in the production of Hippuric acid. “Hippuric acid is a carboxylic acid and organic compound formed from the combination of benzoic acid and glycine. It is primarily found in urine and its levels can increase with the consumption of phenolic compounds, such as those found in fruit juice, tea, and wine. Hippuric acid is also significant in various fields, including cell biology and nutrition, and is used to study metabolism and urinary excretion. Additionally, it is derived from herbivorous animals and plays a role in the body's detoxification processes.”



(–)-Epigallocatechin-3-O-gallate (IF)
(–)-Epigallocatechin (IF)
(–)-Epicatechin (IF)
Pyrogallol (F,U)
Pyrocatechol (F,U)
1-(3',4',5'-Trihydroxyphenyl)-3-(2'',4'',6''-trihydroxy)propan-2-ol*
1-(3',4'-Dihydroxyphenyl)-3-(2'',4'',6''-trihydroxy)propan-2-ol*
5-(3',4',5'-Trihydroxyphenyl)-γ-valerolactone (F,U)
5-(3',4'-Dihydroxyphenyl)-γ–valerolactone (F)
5-(3',4',5'-Trihydroxyphenyl)-valeric acid*
5-(3',4'-Dihydroxyphenyl)-valeric acid (F)
4'-Hydroxyphenylacetic acid (F,U)
3',4'-Dihydroxyphenylacetic acid*
3-(3'-Hydroxyphenyl)propionic acid (F,U)
4-Hydroxybenzoic acid (U)
3'-Methoxy-4'-hydroxyphenylacetic acid (U)
3-(3'-Hydroxyphenyl)hydracrylic acid (U)
Hippuric acid (U)

13.14 “Biochemically, hippuric acid is produced from benzoic acid and glycine, which occurs in the liver, intestine, and kidneys.[5] Hippuric acid has been reported to be a marker for Parkinson's disease.[9]”

13.14.1 Ref. 5: Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA, Peters EC, Siuzdak G (March 2009). "Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites". Proc. Natl. Acad. Sci. U.S.A. 106 (10): 3698–3703. Bibcode:2009PNAS..106.3698W. doi:10.1073/pnas.0812874106. PMC 2656143. PMID 19234110.

13.14.2 Ref. 9: "Parkinson's smell test explained by science". BBC News. BBC. 20 March 2019. Retrieved 11 March 2023.

13.14.3 As a note, a relationship cycle is known as “4-Hydroxybenzoic acid is an aromatic organic acid derived from benzoic acid. It can be produced endogenously through the metabolism of tyrosine, an aromatic amino acid.” (https://www.rupahealth.com/biomarkers/4-hydroxybenzoic-acid)

13.15 “Hippuric acid, or N-benzoylglycine, is an amino acid derivative found in the urine of herbivorous animals. In 1879, Loew wrote about the source of the acid in herbivores’ urine. According to an account of the article in the first volume of the Journal of the American Chemical Society (1879), quinic acid in hay turns into hippuric acid during digestion. (https://www.acs.org/molecule-of-the-week/archive/h/hippuric-acid.html)

13.15.1 “Hippuric acid is present in beef cattle urine and acts as a natural inhibitor of N2O emissions (1,3,5). It reduces the N2O emissions from cattle urine by inhibiting the activities of soil nitrifiers (1).”

13.15.1.1 Ref.(1) Gao J, Zhao G. Potentials of using dietary plant secondary metabolites to mitigate nitrous oxide emissions from excreta of cattle: Impacts, mechanisms and perspectives. Anim Nutr. 2022 Jan 23;9:327-334. doi: 10.1016/j.aninu.2021.12.006. PMID: 35647327; PMCID: PMC9118128.

13.15.1.1.1 Abstract: Nitrous oxide (N2O) is a potent greenhouse gas as well as the key component depleting the ozone sphere of the earth. Cattle have high feed and water intakes and excrete large amounts of urine and feces. N2O can be produced from cattle excreta during storage and use as fertilizer. Mitigating the N2O emissions from cattle excreta during production is important for protecting the environment and the sustainable development of the cattle industry. Feeding cattle with low-protein diets increases N utilization rates, decreases N excretion and consequently reduces N2O emissions. However, this approach cannot be applied in the long term because of its negative impact on animal performance. Recent studies showed that dietary inclusion of some plant secondary metabolites such as tannins, anthocyanins, glucosinolates and aucubin could manipulate the N excretion and the urinary components and consequently regulate N2O emissions from cattle excreta. This review summarized the recent developments in the effects of dietary tannins, anthocyanins and glucosinolates on the metabolism of cattle and the N2O emissions from cattle excreta and concluded that dietary inclusion of tannins or anthocyanins could considerably reduce N2O emissions from cattle excreta.

13.15.1.1.2 Ref.(3) J. Dijkstra, O. Oenema, J.W. van Groenigen, J.W. Spek, A.M. van Vuuren, A. Bannink, Diet effects on urine composition of cattle and N2O emissions, Animal, Volume 7, Supplement 2, 2013, Pages 292-302, ISSN 1751-7311, https://doi.org/10.1017/S1751731113000578. (https://www.sciencedirect.com/science/article/pii/S1751731113000578)

13.15.1.1.2.1 Abstract: Ruminant production contributes to emissions of nitrogen (N) to the environment, principally ammonia (NH_3), nitrous oxide (N_2O) and di-nitrogen (N_2) to air, nitrate (NO_3^-) to groundwater and particulate N to surface waters. Variation in dietary N intake will particularly affect excretion of urinary N, which is much more vulnerable to losses than is faecal N. Our objective is to review dietary effects on the level and form of N excreted in cattle urine, as well as its consequences for emissions of N_2O. The quantity of N excreted in urine varies widely. Urinary N excretion, in particular that of urea N, is decreased upon reduction of dietary N intake or an increase in the supply of energy to the rumen microorganisms and to the host animal itself. Most of the N in urine (from 50% to well over 90%) is present in the form of urea. Other nitrogenous components include purine derivatives (PD), hippuric acid, creatine and creatinine. Excretion of PD is related to rumen microbial protein synthesis, and that of hippuric acid to dietary concentration of degradable phenolic acids. The N concentration of cattle urine ranges from 3 to 20 g/l. High-dietary mineral levels increase urine volume and lead to reduced urinary N concentration as well as reduced urea concentration in plasma and milk. In lactating dairy cattle, variation in urine volume affects the relationship between milk urea and urinary N excretion, which hampers the use of milk urea as an accurate indicator of urinary N excretion. Following its deposition in pastures or in animal houses, ubiquitous microorganisms in soil and waters transform urinary N components into ammonium (NH_4^+), and thereafter into NO_3^- and ultimately in N_2 accompanied with the release of N_2O. Urinary hippuric acid, creatine and creatinine decompose more slowly than urea. Hippuric acid may act as a natural inhibitor of N_2O emissions, but inhibition conditions have not been defined properly yet. Environmental and soil conditions at the site of urine deposition or manure application strongly influence N_2O release. Major dietary strategies to mitigating N_2O emission from cattle operations include reducing dietary N content or increasing energy content, and increasing dietary mineral content to increase urine volume. For further reduction of N_2O emission, an integrated animal nutrition and excreta management approach is required.

13.15.1.2 Ref.(5) Jian Gao, Bingbing Cheng, Yufeng Liu, Meng M. Li, Guangyong Zhao, Dietary supplementation with red cabbage extract rich in anthocyanins increases urinary hippuric acid excretion and consequently decreases nitrous oxide emissions in beef bulls, Animal Feed Science and Technology, Volume 281, 2021, 115075, ISSN 0377-8401, https://doi.org/10.1016/j.anifeedsci.2021.115075. (https://www.sciencedirect.com/science/article/pii/S0377840121002613)

13.15.1.2.1 Abstract: Two consecutive experiments were conducted to investigate the effects of dietary supplementation with red cabbage extract (RCE) rich in anthocyanins on the nitrogen (N) metabolism in beef bulls and the nitrous oxide (N_2O) emissions from the soil applied with the urine of beef bulls. In Experiment 1, 8 Simmental beef bulls (body weight 387.9 ± 40.7 kg) were used as experimental animals. Two levels of RCE, i.e. 0 and 114 g dry matter per day, were supplemented to a basal ration as experimental treatments. The animals and the treatments were allocated in a 2 × 2 crossover design. In Experiment 2, the static incubation technique was used to determine the N2O-N emissions from the soil applied with the urine samples collected from Experiment 1. The results of Experiment 1 indicated that RCE supplementation did not affect the N excretion, N retention, and N retention rate in beef bulls ($P > 0.10$). However, RCE supplementation increased the ratio of hippuric acid-N/urinary N ($P < 0.05$) and tended to increase the ratio of creatinine-N/urinary N ($P = 0.081$) without changing the excretion of other urinary nitrogenous components ($P > 0.10$). Plasma metabolome analysis indicated that RCE supplementation upregulated 12 metabolites while downregulated 18 metabolites ($P < 0.05$). The results of Experiment 2 indicated that RCE supplementation reduced the estimated urine N2O-N emissions by 33.1% through decreasing the ratio of N2O-N/urine-N ($P < 0.05$). In conclusion, RCE supplementation did not affect the N retention and N excretion of beef bulls, whereas it attenuated the urine N2O-N emissions of beef bulls through increasing urinary hippuric acid excretions.

13.15.2 Hence, IFUS finds another plausible biochemical pathway supporting claims by ranchers and dairymen applying SGP+™ as part of their Ration Management Strategy, whereby the claims of pastures devoid of smells of ammonia, manure, and the like replaced with a "somewhat spicy and fresh earth smell," whereas dilute concentrations of N2O can gave a distinct sweet smell.

13.15.2.1 And, the reduction of N2O by Hippuric Acid is linked to 4-Hydroxybenzoic acid, which in turn is found in and/or produced by the active ingredients in SGP+™.

13.15.2.2 “4-Hydroxybenzoic acid supplementation in dairy cow diets may improve milk quality by modifying the fatty acid profile and increasing the bioavailability of polyphenolic compounds. Troubleshooting milk quality issues requires a whole farm approach, including nutrition and feed management, environmental cleanliness, milking parlor prep procedures and maintenance, genetics, and culling decisions.”

13.15.2.3 “Since the (poly)phenols are highly reactive, to overcome these problems, the formulation of a complex of polyphenolic compounds with natural biopolymers is an effective approach. Besides, to increase the bioavailability and bioaccessibility of polyphenolic compounds, milk proteins such as whey protein concentrate, sodium caseinate, and milk protein concentrate act as natural vehicles, due to their specific structural and functional properties with high nutritional value. Therefore, milk proteins are suitable for the delivery of polyphenols to parts of the gastrointestinal tract. Therefore, this review reports on types of (poly)phenols, methods for the analysis of binding interactions between (poly)phenols–milk proteins, and structural changes that occur during the interaction.” (1) Lignin would be one such natural biopolymer as would be other natural biopolymer-like compounds (e.g., waxes, ISP’s, etc.) found in Sugarcane Bagasse. Also, “Pistacia lentiscus, also known as Ash, contains natural polymers such as resin, which is a natural polymer found in the plant. The resin has been characterized and found to contain bioactive compounds like flavonoids and phenolics. It has been recognized for its antioxidant activity and potential use in food and cosmetic industries.” (2)

13.15.2.3.1 Ref.(1) Tosif MM, Najda A, Bains A, Krishna TC, Chawla P, Dyduch-Siemińska M, Klepacka J, Kaushik R. A Comprehensive Review on the Interaction of Milk Protein Concentrates with Plant-Based Polyphenolics. Int J Mol Sci. 2021 Dec 17;22(24):13548. doi:

10.3390/ijms222413548. PMID: 34948345; PMCID: PMC8709213.

13.15.2.3.2 Ref.(2) Vasiliki K. Pachi, Eleni V. Mikropoulou, Petros Gkiouvetidis, Konstantinos Siafakas, Aikaterini Argyropoulou, Apostolis Angelis, Sofia Mitakou, Maria Halabalaki,

13.15.2.3.3 Traditional uses, phytochemistry and pharmacology of Chios mastic gum (Pistacia lentiscus var. Chia, Anacardiaceae): A review, Journal of Ethnopharmacology, Volume 254, 2020, 112485, ISSN 0378-8741, https://doi.org/10.1016/j.jep.2019.112485. (https://www.sciencedirect.com/science/article/pii/S0378874119331174)

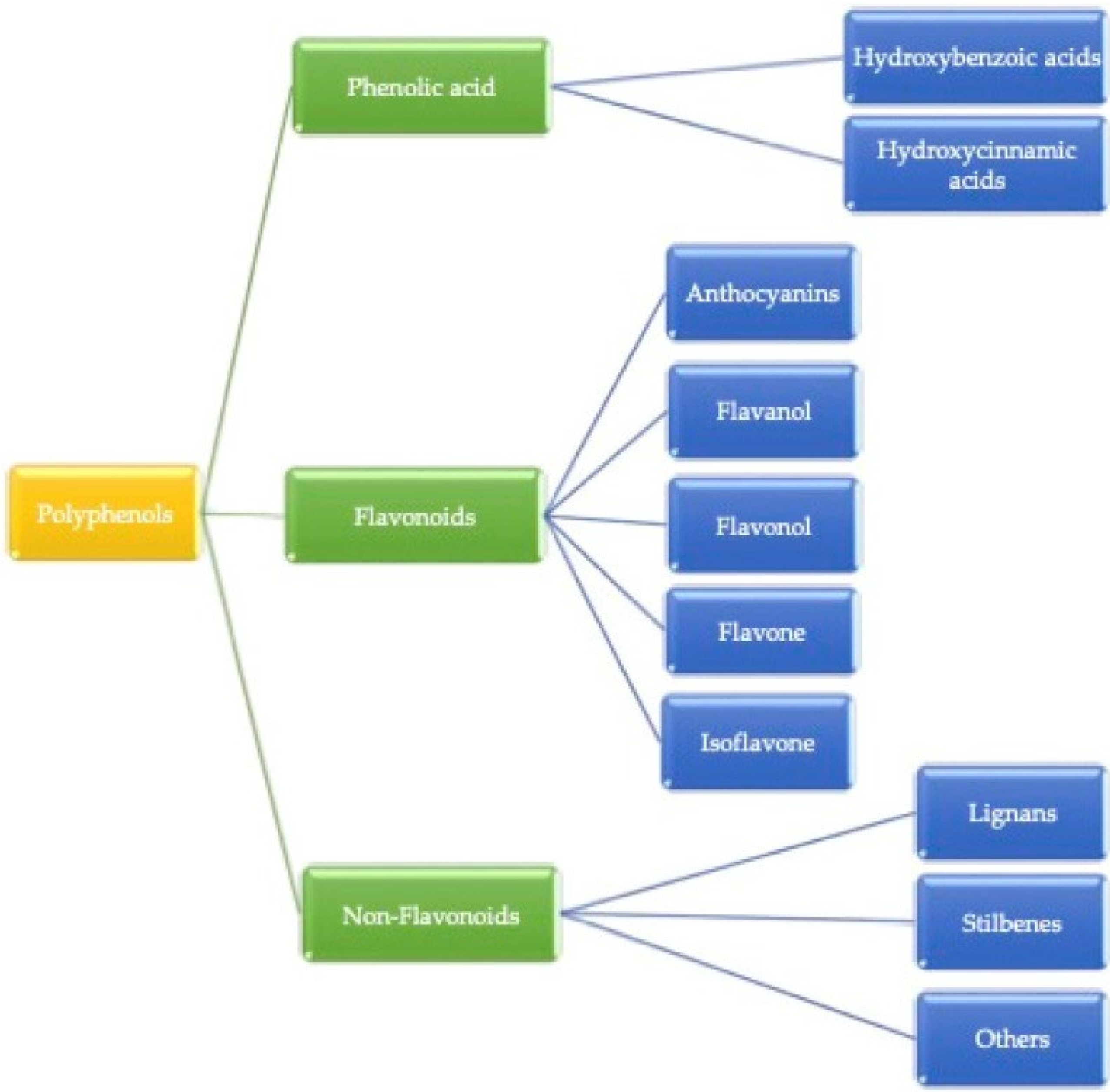
13.15.2.3.3.1 "Abstract: Ethnopharmacological relevance: Chios mastic gum constitutes a unique Greek product, produced exclusively in the southern part of the island of Chios. References about its use from local populations for the treatment of gastrointestinal disorders or as a cosmetic agent can even be encountered in ancient texts of Galen, Theophrastus and Dioscorides. Nowadays, this versatile resin has been rediscovered, not only as a traditional remedy and aromatic agent, but as a potent phytotherapeutic product with various biological properties."

13.15.2.3.4 "4-Hydroxybenzoic acid can affect dairy cows in the following ways (1, 2,3,4): (a) It can increase the solubility of milk proteins. (b) It may cause dysfunction, sickness, or death in animals. (c) It can pass into milk or animal tissues and enter the human food supply. (d) It may cause liver damage and decreased milk production at high levels."

13.15.2.3.4.1 Where Items (a)and (b) produce beneficial effects in the dairy cow, Items (c) and (d) reflect the imbalance or improper metabolism of 4-Hydroxybenzoic acid. This is true for virtually any natural or synthetic ingredient. Hence, rather than throwing a supplement or some synthetic ingredient at the cow, why not attempt to create and integrated ration mix with natural ingredients that works with forage and some minimal level of grain (e.g., cracker corn) to produced eco-friendly, cost-effective, and healthy Herd Performance

13.15.2.3.4.1.1 Ref.(1): In a study published by Tosif MM, et.al., "A Comprehensive Review on the Interaction of Milk Protein Concentrates with Plant-Based Polyphenolics." (Int J Mol Sci. 2021 Dec 17;22(24):13548. doi: 10.3390/ijms222413548. PMID: 34948345; PMCID: PMC8709213.), an exert from the "Abstract" states, "Besides, to increase the bioavailability and bioaccessibility of polyphenolic compounds, milk proteins such as whey protein concentrate, sodium caseinate, and milk protein concentrate act as natural vehicles, due to their specific structural and functional properties with high nutritional value. Therefore, milk proteins are suitable for the delivery of polyphenols to parts of the gastrointestinal tract. Therefore, this review reports on types of (poly)phenols, methods for the analysis of binding interactions between (poly)phenols–milk proteins, and structural changes that occur during the interaction.)

13.15.2.3.5 In “FIGURE 1: Classification of (poly)phenols”, and the subsequent discussions to follow,, we find value-added information like “Moreover, the binding of (poly)phenolic compounds and milk proteins greatly influence the digestibility of proteins, the bioavailability of (poly)phenols, and essential amino acids. Besides, the positive aspects of milk protein (poly)phenolic complexes and commercialization of the food product are important.”



13.15.2.3.6 (Ref.2) We find added support for this information in work published by Zeb, A. (2021). "Phenolic Antioxidants in Dairy Products. In: Phenolic Antioxidants in Foods: Chemistry," (Biochemistry and Analysis. Springer, Cham. https://doi.org/10.1007/978-3-030-74768-8_10). Again, an exert from the "Abstract" states. "Milk obtained from goats, cows, buffaloes, and camels is considered a complete diet of having all the required nutrients. Phenolic compounds in milk originated from the animal feed, whereas supplementation is carried out in other dairy products for attaining the desired taste, odor, and stability. The phenolic compounds present in dairy products include simple phenols, hydroxybenzoic acids, hydroxycinnamic acids, flavonoids, and anthocyanidins. The applications of these phenolic compounds have been discussed."

13.15.2.3.7 In Ref.(3), we find guidance on plants and specific compounds that when improperly used, digested, and absorbed prove detrimental to both beef and dairy cows. ("Nutrient Requirements of Dairy Cattle: Eighth Revised Edition." National Academies of Sciences, Engineering, and Medicine; Division on Earth and Life Studies; Board on Agriculture and Natural Resources; Committee on Nutrient Requirements of Dairy Cattle.) Washington (DC): National Academies Press (US); 2021 Aug 30. To date, not a single negative effect of SGP+™ has been reported in application to beef and dairy heifers, calves, and steers. At this time SGP+™ is NOT recommended for breeding bulls.

13.15.2.3.8 In Ref.(4), we find. "Mycotoxin Effects on Dairy Cattle," by Bill Seglar, DVM, PAS, Nutritional Sciences Manager, Pioneer Hi-Bred Intl., Inc., Box 1150, Johnston, IA 50131-1150, Team Forage, Division of Extension, University of Wisconsin-Madison. To date, not a single negative effect of SGP+™ has been reported in application to beef and dairy heifers, calves, and steers. At this time SGP+™ is NOT recommended for breeding bulls.

13.16 In two studies, certain biting flies have been linked to bovine mastitis (1, 2).

13.16.1 Ref.(1) Biting Flies on Dairy Farms Can Spread Bovine Mastitis, Press Release from American Society of Microbiology, June 26, 2024.

13.16.2 Highlights: Bovine mastitis is a potentially fatal condition with myriad known causes, including bacteria.

1. Biting flies may help cause mastitis, but the mechanisms are not well elucidated.

2. Researchers characterized microbial diversity in biting flies and manure to look for connections.

3. The flies carried relevant bacterial strains, also found in the manure, associated with mastitis.

4. The research may point to new strategies for protecting cows from disease-causing pathogens.

13.16.2.1 Ref.(2) Genetic analysis confirms stable flies as mastitis vector: Research shows flies can carry the bacteria from manure breeding sites, Stew Slater, July 22, 2024, Farmtario: Cow health, Dairy, https://farmtario.com/dairy/cow-health/genetic-analysis-confirms-stable-flies-as-mastitis-vector/

13.17 Natural compounds found and/or metabolized from plants in healthy concentrations in the beef and dairy cows (like 4-Hydroxybenzoic acid) have been linked in numerous studies to fly and overall insect repellency. This would seem to be particularly true if these compounds are found in milk. While creating healthy benefits for humans, they might as well offer a level of protection to the cow.

13.17.1 ADD SOURCES

14.0 Furthermore, 4-Hydroxybenzoic acid demonstrates the capacity to prevent bad fungi from impacting healthy plants:

14.1 Maniak H, Matyja K, Pląskowska E, Jarosz J, Majewska P, Wietrzyk J, Gołębiowska H, Trusek A, Giurg M. 4-Hydroxybenzoic Acid-Based Hydrazide-Hydrazones as Potent Growth Inhibition Agents of Laccase-

Producing Phytopathogenic Fungi That Are Useful in the Protection of Oilseed Crops. Molecules. 2024 May 8;29(10):2212.

14.2 The aforementioned study goes on to demonstrate that "4-Hydroxybenzoic acid can be used to:

14.2.1 Promote plant growth and development.

14.2.2 Increase abiotic stress tolerance, such as drought and freezing tolerance.

14.2.3 Improve the economics of biorefineries when accumulated in sorghum biomass.

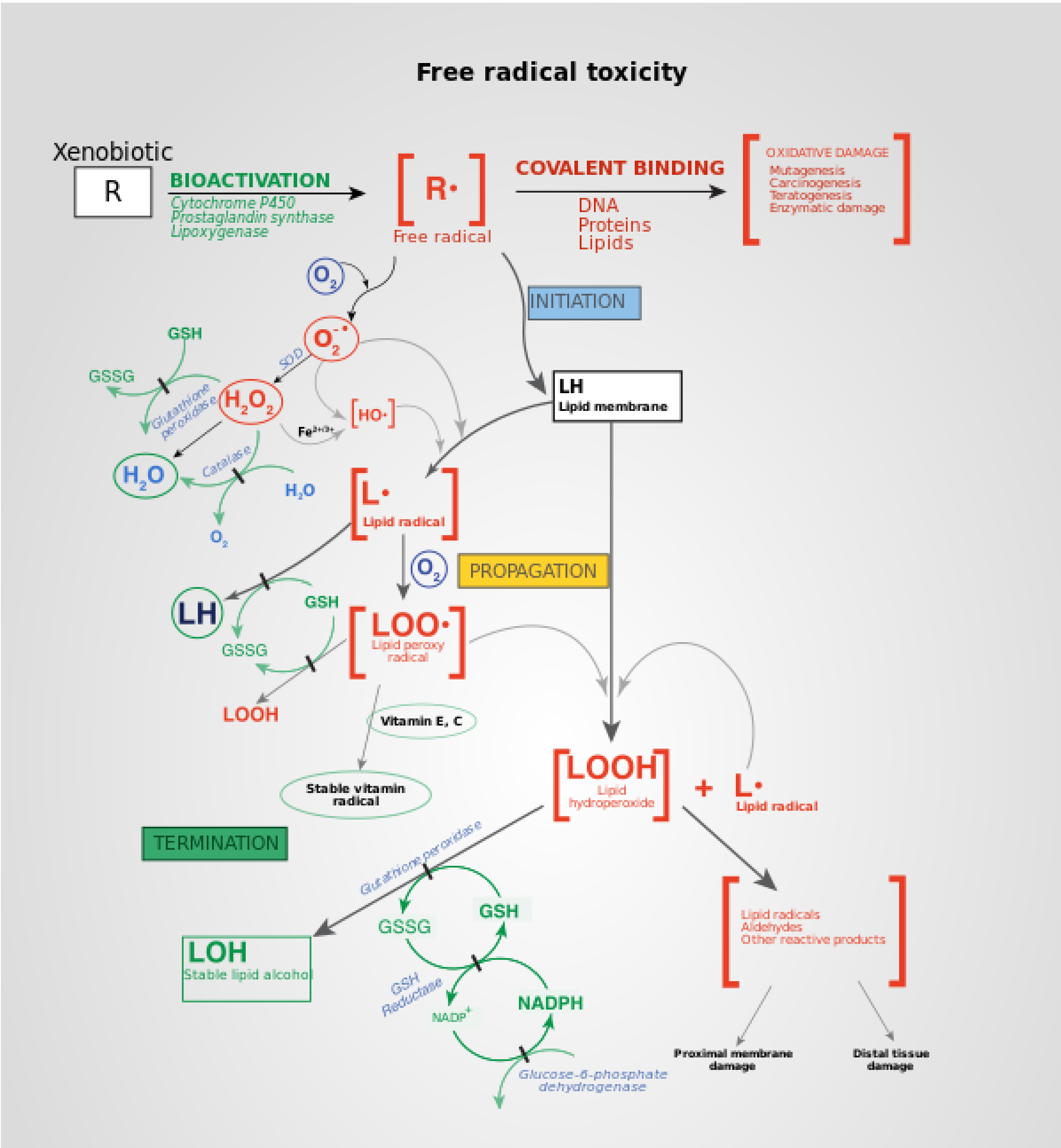
14.2.4 Accumulate in phloem fluids and be involved in plant defense."

15.0 Flavonoids:

15.1 The results obtained in the present meta-analysis indicate that FLAs can be used as natural growth promoters in beef cattle and, at the same time, improve feed conversion. The best result for daily weight gain is obtained with FLAs supplementation periods up to 75 days and diets high in concentrate (>700 g/kg DM). Likewise, including FLAs in bovine diets improves dry matter intake and nutrient digestibility. The best dry matter intake is obtained with periods up to 75 days and when the FLAs used are puerarin, anthocyanin, and daidzein. Furthermore, supplementation with FLAs improves total antioxidant status and immune response in cattle by reducing serum concentration of malondialdehyde and increasing serum levels of antioxidant enzymes and immunoglobulins. The best results for serum concentration of superoxide dismutase are obtained with FLAs extracts and when the FLAs used are puerarin or daidzein. At the same time, FLAs supplementation improves meat quality by reducing shear force and malondialdehyde content. In addition, FLAs improve milk production and composition. The highest milk production is obtained when FLAs extracts are used, with daidzein or mixtures of FLAs, and low doses of FLAs (≤600 mg/kg DM). The best results for milk protein content are obtained with supplementation periods longer than 75 days, diets with moderate levels of concentrate (400–700 g/kg DM), and daidzein or mixtures of FLAs. Likewise, the best fat content in milk is achieved with daidzein or mixtures of FLAs and using cows with more than 100 days in milk. Finally, FLAs supplementation improves ruminal fermentation in cattle through increased ruminal propionate concentration and reduced total rumen protozoa. The best rumen propionate concentration is obtained with supplementation periods of up to 75 days.

15.1.1 Orzuna-Orzuna JF, Dorantes-Iturbide G, Lara-Bueno A, Chay-Canul AJ, Miranda-Romero LA, Mendoza-Martínez GD. Meta-analysis of flavonoids use into beef and dairy cattle diet: Performance, antioxidant status, ruminal fermentation, meat quality, and milk composition. Front Vet Sci. 2023 Feb 15;10:1134925. doi: 10.3389/fvets.2023.1134925. PMID: 36876000; PMCID: PMC9975267.

Free Radical Toxicity & The Role of Antioxidants:



Free radical mechanisms in tissue injury. Lipid peroxidation induced by xenobiotics and the subsequent detoxification by cellular enzymes (termination).

Fractals 1: A fractal is a complex geometric shape that exhibits self-similarity across different scales, meaning that its structure is repeated at various levels of magnification. Fractals are often described as never-ending patterns created by repeating a simple process in a recursive manner, resulting in infinitely complex designs. Mathematically, fractals can have a fractal dimension that exceeds their topological dimension, distinguishing them from traditional geometric shapes like squares and circles. They are used in various fields, including mathematics, nature, and art, to model complex structures and phenomena.

Fractals 2: Fractals in mitochondria are characterized by their multifractal structure, which is influenced by features such as loops, hairpins, and inverted palindromes in the mitochondrial DNA genome1. This self-similarity is linked to the function of subsequences, establishing a relationship between their fractal dimension and structure1. Additionally, dynamic fractals in mitochondria express long-term correlation and intrinsic coordination, indicating a broad range of oscillatory frequencies3. Techniques like MitoMorF combine automated mitochondrial morphology with fractal analysis to discern subtle changes in mitochondrial dynamics4.

Addendum1: What do we think we know about Mastic:

Since the dawn of recorded history, "mastic" has been cited for its nutritive and healing value.

Beneficial effects of Mastic on the health of the bovine should include:

- Healing of lesions and ulcerations on the surfaces of the mouth, the reticulum, the rumen, the omasum, the abomasum, the intestines, the liver, the kidney and the pancreas.
- Reduction and / or elimination of the *Helicobacter pylori* bacteria in the mouth, the reticulum, the rumen, the omasum, the abomasum, the intestines, the liver, the kidney and the pancreas.
- Improved reproductive performance

- "Data reviewed shows that supplementation with different sources of lipids and fatty acids improve reproductive performance of the female ruminant. However, it is important to consider that the optimum response will be achieved when under-nutrition status of the female is not extremely severe. A nutrient balance (protein: energy) in the ration consumed by the animal is fundamental to obtain maximum benefit from supplementation with fat, since fatty acids do not supply nitrogen for amino acid synthesis and consequently for the correct functioning of the hypothalamus-hypophysis axis. Improvements in reproductive performance may be a result of increased energy density of the ration or of the direct effects of specific fatty acids on reproductive processes. As is the case for any technology or management strategy that improves specific aspects of ovarian physiology and cyclic activity, actual improvements in pregnancy rate or total weight of calf weaned are dependent on a variety of management practices and environmental conditions.
- Until these interrelationships are better understood, livestock producers are recommended to attempt to formulate low cost/balanced rations. If a source of supplemental fat is available locally and can be incorporated with little or no change in the cost of the ration, it would be wise for farmers to do so. Research studying the role of fat supplementation on reproductive responses has not been that consistent, therefore, adding fat to the ration would be advised when the risk of low reproductive performance (young, growing animals and limiting nutrients [protein, energy] in the basal ration) is the greatest.
    - Effect of Fatty Acids on Reproductive Performance of Ruminants, Herrera-Camacho, José1, Soberano-Martínez, Alejandra1, Orozco Durán, Karlos Edmundo2, Aguilar-Pérez, Carlos2 and Ku-Vera, Juan Carlos2, *1Instituto de Investigaciones Agropecuarias y Forestales Universidad Michoacana de San Nicolás de Hidalgo, 2Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, Artificial Insemination in Farm Animals, www.intechopen.com*

- Improved lactation and estrogenic balance have also been suggested by cattle fed Mastic.

Mastic (Greek: Μαστίχα) is a resin obtained from the mastic tree (*Pistacia lentiscus*). In pharmacies and nature shops, it is called "arabic gum" (not to be confused with gum arabic) and "Yemen gum".

- In Greece, it is known as the "tears of Chios," being traditionally produced on that Greek island, and, like other natural resins, is produced in "tears" or droplets.

- Originally liquid, it is sun dried into drops of hard brittle translucent resin.
- When chewed, the resin softens and becomes a bright white and opaque gum.
- The flavor is bitter at first, but after chewing, it releases a refreshing, slightly piney or cedar flavor.

"Tschirch and Reutter (*A. Pharm*., 1904, 104) examined mastic carefully:

- They found an ethereal oil, possessing a pale yellow color and a somewhat camphoraceous odor.
    - There is also a bitter principle, which could not be isolated in pure form."
- It is worthy to note that a "bitter principle" is basic qualitative chemical analysis is typically noted as being a "base" versus an "acid" as bases are inherently bitter.

One of the key ingredients of Mastic is Gallic acid ($C_6H_2(OH)_3COOH$). It is:

- a trihydroxybenzoic acid
- a type of phenolic acid
- a type of organic acid, also known as 3,4,5-trihydroxybenzoic acid.
- found both free and as part of hydrolysable tannins.

Gallic Acid is an active ingredient in mastic and is thought to have positive impacts on protein digestibility and nitrogen utilization in the rumen in the form of:

- Pyrogallol, a hepatotoxin and nephrotoxin, is a product of hydrolyzable tannins (HT) degradation by ruminal microbes.
- Proanthocyanidins (PA) (condensed tannins) are considered to be non-toxic because they are not absorbed, but they are associated with lesions of the gut mucosa.
- Research on tannins in forage legumes has determined their effects on protein digestion and metabolism but more research on tannin structure in relation to digestion of specific proteins is needed.
- The widely accepted explanation for positive effects of PA on protein digestion and metabolism is that PA-protein complexes escape ruminal degradation and the protein is available in the lower tract.

- This proposed mechanism may be incorrect because PA also complex carbohydrates, endogenous proteins, and microbial products and the degradability of PA-protein complexes by ruminal microbes has not been adequately studied.
- Several alternative hypotheses (to escape protein) that explain the effect of PA on protein digestion and metabolism in ruminants are also consistent with experimental results on forage legumes.
- These include increased microbial protein synthesis, increased use of endogenous nitrogen in the rumen, and increased secretion of salivary glycoproteins.
- Research on manipulating the content and type of PA in forage legumes is justified because they are associated with non-bloating legumes, lower soluble non-protein nitrogen in silage, and improved efficiency of protein utilization.
    - Jess D Reed, Nutritional Toxicology Polyphenols in Tannins and Related Forage Legumes, Volume: 73, Issue: 5, Pages: 1516-1528, Journal of Animal Science (1995)
- NOTE: Carob is also legume.

Gallic acid is:

- commonly used in the pharmaceutical industry
    - S. M. Fiuza. "Phenolic acid derivatives with potential anticancer properties—a structure–activity relationship study. Part 1: Methyl, propyl and octyl esters of caffeic and gallic acids". Elsevier. doi:10.1016/j.bmc.2004.04.026.
- a standard for determining the phenol content of various analytes by the Folin-Ciocalteau assay
    - Andrew Waterhouse. "Folin-Ciocalteau Micro Method for Total Phenol in Wine". UC Davis.
- a starting material in the synthesis of the psychedelic alkaloid mescaline
    - Tsao, Makepeasce (July 1951). "A New Synthesis Of Mescaline". *Journal of the American Chemical Society* **73** (11): 5495–5496. doi:10.1021/ja01155a562. ISSN 0002-7863.

Mastic has been used as a medicine since antiquity and is still used in traditional folk medicine of the Middle East.

- In ancient Greece, it was given as a remedy for snakebite, and, in India and Persia, it was used to fill dental cavities. The first-century Greek physician Pedanius Dioscorides mentions the healing properties of mastic in his book *De Materia Medica*.

- Hippocrates wrote that the mastic is good for prevention of digestive problems and colds.
- Galenus suggested that mastic was useful for bronchitis and for improving the condition of the blood.
- In medieval times, mastic was highly valued by sultans' harems as a breath freshener and a tooth whitener.

Commonly held benefits of mastic include:

- Several trace components that appear to contribute significantly to the antibacterial activity of mastic oil have been identified: verbenone, alpha-terpineol, and linalool. The sensitivity to these compounds was different for different bacteria tested (Escherichia coli, Staphylococcus aureus, and Bacillus subtilis), which suggests that the antibacterial efficacy of mastic oil is due to a number of its components working synergistically.
    - J Agric Food Chem. 2005 Oct 5; 53(20):7681-5. Chemical composition and antibacterial activity of the essential oil and the gum of Pistacia lentiscus Var. chia., Koutsoudaki C, Krsek M, Rodger A., Department of Chemistry and Department of Biological Sciences, University of Warwick, Warwick, Coventry CV4 7AL, United Kingdom.
- Gum of Chios mastic (Pistacia lentiscus var. chia) is a natural antimicrobial agent that has found extensive use in pharmaceutical products and as a nutritional supplement.
    - The molecular mechanisms of its anti-inflammatory activity, however, are not clear. In this work, the potential role of antioxidant activity of Chios mastic gum has been evaluated.
    - Scavenging of superoxide radical was investigated by electron spin resonance and spin trapping technique using EMPO spin trap in xanthine oxidase system.
        - Superoxide production in endothelial and smooth muscle cells stimulated with TNF-α or angiotensin II and treated with vehicle (DMSO) or mastic gum (0.1-10 μg/ml) was measured by DHE and HPLC.
        - Cellular H2O2 was measured by Amplex Red. Inhibition of protein kinase C (PKC) with mastic gum was determined by the decrease of purified PKC activity, by inhibition of PKC activity in cellular homogenate and by attenuation of superoxide production in cells treated with PKC activator phorbol 12-myristate 13-acetate (PMA).
    - Spin trapping study did not show significant scavenging of superoxide by mastic gum itself.
        - However, mastic gum inhibited cellular production of superoxide and H2O2 in dose dependent manner in TNF-α treated rat aortic smooth muscle cells but did not affect unstimulated cells.
        - TNF-α significantly increased the cellular superoxide production by NADPH oxidase, while mastic gum completely abolished this stimulation.
        - Mastic gum inhibited the activity of purified PKC, decreased PKC activity in cell homogenate, and attenuated superoxide production in

cells stimulated with PKC activator PMA and PKC-dependent angiotensin II in endothelial cells.

  - Conclusion: We suggest that mastic gum inhibits PKC which attenuates production of superoxide and H2O2 by NADPH oxidases. This antioxidant property may have direct implication to the anti-inflammatory activity of the Chios mastic gum.
    - Nutr J. 2011 Jun 6;10:64. doi: 10.1186/1475-2891-10-64. Anti-inflammatory activity of Chios mastic gum is associated with inhibition of TNF-alpha induced oxidative stress. Triantafyllou A, Bikineyeva A, Dikalova A, Nazarewicz R, Lerakis S, Dikalov S., Medical School of Athens, Athens, Greece.
- Mastic contains antioxidants and also has antibacterial and antifungal properties.
  - Koutsoudaki C, Krsek M, Rodger A (October 2005). "Chemical composition and antibacterial activity of the essential oil and the gum of Pistacia lentiscus Var. chia". *Journal of Agricultural and Food Chemistry* **53** (20): 7681–5. doi:10.1021/jf050639s. PMID 16190616.
- A Nottingham University study published in the *New England Journal of Medicine* claims that mastic can cure peptic ulcers by killing *Helicobacter pylori* bacteria.
  - (Huwez FU, Thirlwell D, Cockayne A, Ala'Aldeen DA (December 1998). "Mastic gum kills Helicobacter pylori". *The New England Journal of Medicine* **339** (26): 1946. doi:10.1056/NEJM199812243392618. PMID 9874617.)
- Other studies have indicated that mastic has only a modest ability to eliminate *H. pylori* but have also suggested that refining mastic by removing the polymer poly-β-myrcene may make the active components, particularly isomasticadienolic acid, more available and effective·
  - Paraschos S, Magiatis P, Mitakou S, *et al.* (February 2007). "In vitro and in vivo activities of Chios mastic gum extracts and constituents against Helicobacter pylori". *Antimicrobial Agents and Chemotherapy* **51** (2): 551–9. doi:10.1128/AAC.00642-06. PMC 1797732. PMID 17116667.
- Mastic may also have some value in preventing tooth decay and gingivitis as chewing mastic reduces oral bacteria.
  - Aksoy A, Duran N, Koksal F (June 2006). "In vitro and in vivo antimicrobial effects of mastic chewing gum against Streptococcus mutans and mutans streptococci". *Archives of Oral Biology* **51** (6): 476–81. doi:10.1016/j.archoralbio.2005.11.003. PMID 16343417.
  - Takahashi K, Fukazawa M, Motohira H, Ochiai K, Nishikawa H, Miyata T (April 2003). "A pilot study on antiplaque effects of mastic chewing gum in the oral cavity". *Journal of Periodontology* **74** (4): 501–5. doi:10.1902/jop.2003.74.4.501. PMID 12747455.
- One study found that high consumption of Chios mastic powder results in decreased levels of total serum cholesterol, LDL, total cholesterol/HDL ratio, lipoprotein (a), apolipoprotein A-1, apolipoprotein B, SGOT, SGPT and gamma-GT.
  - Triantafyllou, A.; Chaviaras, N.; Sergentanis, T. N.; Protopapa, E.; Tsaknis, J. (2007). "Chios mastic gum modulates serum biochemical parameters in a human population".

Journal of Ethnopharmacology **111** (1): 43–49. doi:10.1016/j.jep.2006.10.031. PMID 17150319.

- Various studies also show mastic is used in the manufacture of adhesive bandages, acts as an antioxidant and helps to protect human cells against oxidative damage, to show cytotoxicity against cancer cells, without harming healthy cells, used as a remote astringent in cases of internal hemorrhage, used to treat albuminuria and diabetes, used to treat psoriasis and external hemorrhoids.

Does pistacia lentiscus contain carotenoids? Pistacia lentiscus contains carotenoids, including lutein, zeaxanthin, and β-carotene (1,2,3,4,5).

Ref. (1 & 4) Identification and quantitation of tocopherols, carotenoids and triglycerides in edible Pistacia lentiscus oil from Tunisia, F. Mezni, et.al, . Mater. Environ. Sci., 2020, Volume 11, Issue 1, Page 79-84,

Ref. (2) Floris S, Di Petrillo A, Pintus F, Delogu GL. Pistacia lentiscus: Phytochemistry and Antidiabetic Properties. Nutrients. 2024 May 27;16(11):1638. doi: 10.3390/nu16111638. PMID: 38892571; PMCID: PMC11174566.

Ref. (3) Bouakline H, Bouknana S, Merzouki M, Ziani I, Challioui A, Bnouham M, Tahani A, El Bachiri A. The Phenolic Content of Pistacia lentiscus Leaf Extract and Its Antioxidant and Antidiabetic Properties. ScientificWorldJournal. 2024 Feb 7;2024:1998870. doi: 10.1155/2024/1998870. PMID: 38356989; PMCID: PMC10866636.

Ref. (5) Ghzaiel I, Zarrouk A, Nury T, Libergoli M, Florio F, Hammouda S, Ménétrier F, Avoscan L, Yammine A, Samadi M, Latruffe N, Biressi S, Levy D, Bydlowski SP, Hammami S, Vejux A, Hammami M, Lizard G. Antioxidant Properties and Cytoprotective Effect of Pistacia lentiscus L. Seed Oil against 7β-Hydroxycholesterol-Induced Toxicity in C2C12 Myoblasts: Reduction in Oxidative Stress, Mitochondrial and Peroxisomal Dysfunctions and Attenuation of Cell Death. Antioxidants (Basel). 2021 Nov 5;10(11):1772. doi: 10.3390/antiox10111772. PMID: 34829643; PMCID: PMC8615043.

Addendum 2: What do we think we know about Carob

In ancient times, the Carob tree and the Mastic tree were called “sister trees”.

Ceratonia siliqua, commonly known as the **Carob tree** and **St John's-bread**, is a species of flowering evergreen shrub or tree in the pea family, ***Fabaceae***.

- The seed pod may be crushed and used as ersatz chocolate.
- While previously not believed to form nitrogen fixation nodules typical of the legume family, trees have been identified more recently with nodules containing bacteria believed to be from the *Rhizobium* genus.
    - M. Missbah El Idrissi, N. Aujjar, A. Belabed, Y. Dessaux, A. Filali-Maltouf (1996). "Characterization of rhizobia isolated from Carob tree (Ceratonia siliqua)". *Journal of Applied Microbiology* **80** (2): 165–73. doi:10.1111/j.1365-2672.1996.tb03205.x.

The impact of Carob on beef and dairy cattle is thought to include:

- An impact on the estrogenic cycle and lactation much like cacao in humans.
    - Carob, like cacao, is thought to trigger an endorphin response that relaxes mammalian physiology and increases the release of pheromones.

Nutritional value of Carob includes (The Gilead Institute: Carob Better than Chocolate):

- as much Vitamin B1 as asparagus or strawberries
- as much niacin as lima beans, lentils, or peas
- more Vitamin A than eggplant, asparagus, and beets.
- contains Vitamin B2, calcium, magnesium, potassium, and the trace minerals iron, manganese, chromium, copper, and nickel.
- contains approximately 8 percent protein and is a good source of fiber
- Compared to chocolate, carob is three times richer in calcium, has one third less calories and seventeen times less fat.

- 1 ounce of carob powder has about 100 calories, which is about 1/4 cup in measure.
    - This amount has about 10 grams of fiber and 0.18 grams (virtually none) of fat.
    - It also contains B vitamins, magnesium, iron, manganese, chromium, and copper.
    - It takes 2 medium/large dry carob pods to make 1 ounce in weight.

Carob also has therapeutic uses (The Gilead Institute: Carob Better than Chocolate):

- It is known to halt serious cases of diarrhea in adults, infants, and animals.
- It is also known to help with nausea, vomiting, and upset stomach.
- French physician successfully reversed kidney failure with carob.
- A decoction of the leaves and bark has been useful for syphilis and venereal diseases, and seems to have a soothing effect on epilepsy.

Ceratonia siliqua, the scientific name of the carob tree, derives from the Greek kerátion (κεράτιον), "fruit of the carob" (from *keras* [κέρας] "horn"), and Latin *siliqua* "pod, carob."

- The term "carat", the unit by which gem weight is measured, is also derived from the Greek word kerátion (κεράτιον), alluding to an ancient practice of weighing gold and gemstones against the seeds of the carob tree by people in the Middle East.
- The system was eventually standardized, and one carat was fixed at 0.2 grams.

In late Roman times, the pure gold coin known as the solidus weighed 24 carat seeds (about 4.5 grams).

- As a result, the carat also became a measure of purity for gold. Thus 24-carat gold means 100% pure, 12-carat gold means the alloy contains 50% gold, etc.
    - Harper, Douglas. "carat". *Online Etymology Dictionary*.

Subsistence on carob pods is mentioned in the Talmud: Berakhot reports that Rabbi Haninah subsisted on carob pods.

- It is probably also mentioned in the New Testament, in which Matthew 3:4 reports that John the Baptist subsisted on "locusts and wild honey"; the Greek word translated "locusts" may refer to carob pods, rather than to grasshoppers.
    - http://www.forward.com/articles/a-brief-on-bokser/
- Again, in Luke 15:16, when the Prodigal Son is in the field in spiritual and social poverty, he desires to eat the pods that he is feeding to the swine because he is suffering from starvation.
- The use of the carob during a famine is likely a result of the carob tree's resilience to the harsh climate and drought. During a famine, the swine were given carob pods so that they would not be a burden on the farmer's limited resources.
- During the Second World War, it was common for the people of Malta to eat dried carob pods and prickly pears as a supplement to rationed food.

Carob consumed by humans is the dried (and sometimes roasted) pod, and not the 'nuts' or seeds.

- Carob is mildly sweet and is used in powdered, chip, or syrup form as an ingredient in cakes and cookies, and as a substitute for chocolate.
- Crushed pods may be used to make a beverage; compote, liqueur, and syrup are made from carob in Turkey, Malta, Portugal, Spain and Sicily.
- Several studies suggest that carob may aid in treating diarrhea in infants.
    - Fortier D, Lebel G, Frechette A (June 1953). "Carob flour in the treatment of diarrhoeal conditions in infants". *Canadian Medical Association Journal* **68** (6): 557–61. PMC 1822828. PMID 13059705.
- In Libya, carob syrup (there called *rub*) is used as a complement to Asida.

The seeds, also known as *locust beans*, are used as animal feed, and are the source of locust bean gum — a food thickening agent.

- Chocolate contains theobromine, which is poisonous to some mammals, but carob does not, and is used to make chocolate-flavored treats for dogs.
    - Burg, Barbara. *Good Treats For Dogs Cookbook for Dogs: 50 Home-Cooked Treats for Special Occasions*. Quarry Books, 2007, p. 28

Carob is rich in sugars - Sucrose = 531g ± 93 g/kg dry weight for cultivated varieties and 437 ± 77 g/kg in wild type varieties.

- http://www.sciencedirect.com/science/article/pii/S0308814605010824

Additionally, in "Botanical, Chemical, and Pharmacological Characteristics of Carob Tree (Ceratonia Siliqua L)," (Rahal El Kahkahi, et.al., Med Discoveries. 2024; 3(6): 1168.), Carob is found to possess the following photochemistry:

"The pulp is widely used either as a dietary supplement, a substitute for chocolate or even in animal feed. It is very rich in sugar (48-56%) [19], in particular sucrose, glucose, fructose and maltose, but low in protein (2-6%) and lipids (0.4-0.6%) including fatty acids saturated and unsaturated are in equal proportions [27]. From pod extract, five amino acids, namely alanine, glycine, leucine, proline and valine, have been isolated by [23] and two other compounds, tyrosine and phenylamine, have been reported by [31]. In addition, the pulp also has a very high fiber content (20- 50%) and a significant amount of tannin (18-20%) [28]. In addition, the mineralogical analysis carried out on the pulp revealed a composition (in mg/100 g of the pulp) of: K=1110, Ca=307, Mg=42, Na=13, Cu=0.23, Fe=104, Mn=0.4 and Zn=0.59 [27]."

"The seed is composed of 30 to 33% integumentary envelope, 42 to 46% of the albumen and 23 to 25 of the embryo. The integumentary envelope is considered to be a natural source for the production of antioxidant polyphenol [29]. The albumen consists essentially of gum or galactomannan [30], which is a polysaccharide molecule composed of two sugar units, mannose and galactose, in a ratio of 4:1. This natural polysaccharide is endowed with various important properties, namely high viscosity in water even at variable temperature and pH, ability to form from very dilute solution of stable viscous solution and high potentiality to react with other polysaccharides thus inducing a synergistic effect [28]."

Ref (19) Biner B, Gubbuk H, Karhan M, Aksu M, M Pekmezci. Sugar profiles of the pods of cultivated and wild types of carob bean (Ceratonia siliqua L.) in Turkey. Food Chemistry. 2007; 100: 1455.

Ref (27) Youssef MKE, El-Manfaloty MM, HM Ali. Assessment of proximate chemical compossition, nutritional status, fatty acid composition and phenolic compounds of carob (Ceratonia siliqua L.). Food Public Health. 2013; 304-308.

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Does ceratonia siliqua l. contain carotenoids? Ceratonia siliqua L., commonly known as carob, contains carotenoids (1,2,3,4). These carotenoids include compounds such as lutein, lycopene, carotene, and β-carotene (4).

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Addendum 3: Most common plant-based Phenolic Acids per Robbins, Rebecca. (2003). Phenolic Acids in Foods: An Overview of Analytical Methodology. Journal of agricultural and food chemistry. 51. 2866-87. 10.1021/jf026182t. Phenolic acids are aromatic secondary plant metabolites, widely spread throughout the plant kingdom. Existing analytical methods for phenolic acids originated from interest in their biological roles as secondary metabolites and from their roles in food quality and their organoleptic properties. Recent interest in phenolic acids stems from their potential protective role, through ingestion of fruits and vegetables, against oxidative damage diseases (coronary heart disease, stroke, and cancers). High performance liquid chromatography (HPLC) as well as gas chromatography (GC) are the two separation techniques reviewed. Extraction from plant matrixes and cleavage reactions through hydrolysis (acidic, basic, and enzymatic) are discussed as are the derivatization reagents used in sample preparation for GC. Detection systems discussed include UV-Vis spectroscopy, mass spectrometry, electrochemical, and fluorometric detection. The most common tandem techniques are HPLC/UV and GC/MS, yet LC/MS is becoming more common. The masses and MS fragmentation patterns of phenolic acids are discussed and tabulated as are the UV absorption maxima.

R_2	R_3	R_4	R_5	X	code	common name
H	H	H	H	a	**1**	cinnamic acid
–OH	H	H	H	a	**2**	*o*–coumaric acid
H	H	–OH	H	a	**3**	*p*–coumaric acid
H	–OH	H	H	a	**4**	*m*–coumaric acid
H	$-OCH_3$	–OH	H	a	**5**	ferulic acid
H	$-OCH_3$	–OH	$-OCH_3$	a	**6**	sinapic acid
H	–OH	–OH	H	a	**7**	caffeic acid
H	H	H	H	b	**8**	benzoic acid
–OH	H	H	H	b	**9**	salicylic acid
H	H	–OH	H	b	**10**	*p*–hydroxybenzoic acid
H	$-OCH_3$	–OH	H	b	**11**	vanillic acid
H	$-OCH_3$	–OH	$-OCH_3$	b	**12**	syringic acid
H	–OH	–OH	H	b	**13**	protocatechuic acid
–OH	H	H	–OH	b	**14**	gentisic acid
–OH	–OH	–OH	–OH	b	**15**	gallic acid
H	$-OCH_3$	$-OCH_3$	H	b	**16**	veratric acid
H	$-OCH_3$	–OH	$-OCH_3$	c	**17**	syringealdehyde
H	$-OCH_3$	–OH	H	c	**18**	vanillin