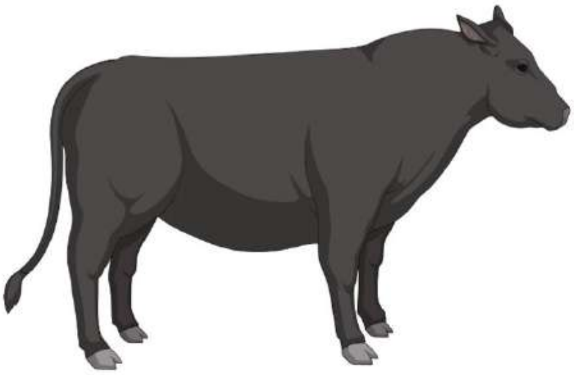
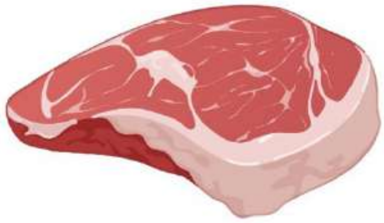
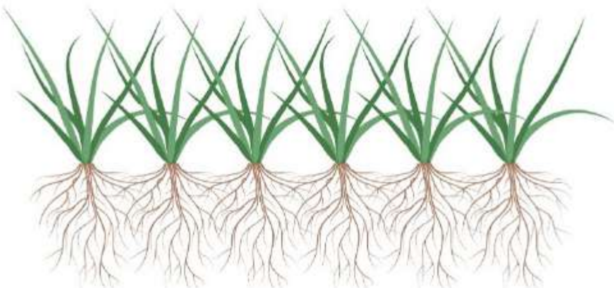
BEEF NUTRIENT DENSITY

Data Report







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Commentary

Growing consumer interest in grass-fed meat has raised several questions about differences in the nutritional quality of beef from grass-fed and grain-fed cattle; however, in-depth nutritional analysis is still needed. We analyzed over 800 unique nutrients/metabolites in your samples including phytochemicals, fatty acids, vitamins, and various markers of animal health in the samples. This represents a much larger nutritional profiling of compounds, which goes far beyond omega-3 fatty acids.

Phytochemicals are naturally occurring compounds derived from plants that could have anti-oxidant and anti-inflammatory effects in animals and humans. These compounds are typically rich in fresh forages and are lower in dried hay/silage and grains. The total **phytochemical** amount **in the samples** was 1.5 times higher than the amount we typically observe in **grass-fed beef samples (slide 6)**. This was driven predominantly by the substantial presence of **N-methylpipecolate (slide 7)** and **stachydrine (slide 8)**, which were **2- and 3-fold higher** than the amounts we typically observe in grass-fed beef, and **8- and 5-fold** higher than the amounts we typically find in grain-fed beef, respectively. These compounds have potentially anti-inflammatory and anti-oxidant effects. These compounds are particularly rich in hay/silage, especially when made from plants in the stachys family (e.g., alfalfa).

Other common phenolics such as hippurate (a major indicator of phenolic intake), thioproline, catechol sulfate, and cinnamoylglycine remained **0.3-0.5 times lower than** the amount we typically find in grass-fed beef samples **(slides 9-16)**. This is likely because the animals were processed in the late fall when grasses were starting to lose quality and/or because the animals may have been consuming some hay, which diminished the phytochemical richness for those specific compounds. Typically, animals fed fresh forages have higher amounts of these compounds, which is related to the presence of these compounds in the forage, which typically are highest in the spring/summer/early fall, but rapidly drop during the early winter months. Processing in Sept/Oct as opposed to Nov/Dec may therefore have benefits.

Grass-fed ruminant meat can be a rich source of omega-3 fatty acids, CLA, and long-chain saturated fatty acids. The samples displayed the most favorable **omega-3 fatty acid, CLA and long-chain saturated** fatty acid profiles **(slides 19-29)** and was similar or more favorable compared to what we typically observe in grass-fed beef samples. The Omega 6:3 ratio was excellent at an average of 1.1 compared to 3.0 for Grass-fed, and 7.8 for Grain-Fed. Higher levels of omega-3 fatty acids in beef can lead to higher levels of these in consumers. Studies associate a higher intake of omega-3 fatty acids with improved brain health and heart health. Fresh forages provide the precursor to Omega-3 fatty acids in meat. **Slide 19** also shows the typical Omega 6:3 ratio in other animal products that are typically fed grains like soybeans and corn, and their values are far higher than the what the HNG beef samples achieved.

The **B-vitamins (slides 33-36)** were reduced compared to the amounts we typically find in grass-fed and grain-fed beef samples. **B-vitamins are** normally produced by bacteria in the rumen on well-balanced roughage diets. B3 and B6 are especially in leguminous forages (barley, triticale), clover, and maize and alfalfa silage in the case of feedstuff. Incorporating clover, oats, peas, triticale and other leguminous forages could be a way to increase these amounts in beef. Since B-vitamins are water-soluble they are not stored in fat and their precursors need to be ingested more regularly. The forage samples from October were also lower in B-vitamins compared to the forage samples from May and July, which represents a logical explanation for the findings in the meat. This information is also in line with the phytochemicals.

Alpha-tocopherol is a vitamin E precursor best known for its anti-oxidative effects. Tocopherols are associated with protection against cardiovascular disease[1], certain cancers, brain function decline, and reduced eye-sight. Typically, alpha-tocopherol is highest in fresh forages, reduced in conserved forages/low quality grasses, and lowest in grain-based concentrates. The alpha-tocopherxol content was **0.4** times the amount we typically find in grass-fed beef samples (Slide 31). Again, fresh forages provide good precursors to this compound.

The mitochondrial metabolites malate and succinate suggest a reliance on oxidative metabolism, which is common in grass-fed animals and implies animals were physically active and on pasture. It also indicates that the animals were in good metabolic health. Markers of oxidative stress and homocysteine **(slides 40-44)** were lower than the average grass-fed and grain-fed samples, further indicating good metabolic health.

Overall, the fatty acid ratios were excellent and we found various phytochemicals, particularly those found in hay/silage, to be enriched in the meat samples. The b-vitamins and alpha-tocopherol (vitamin E) remained lower than we typically observe. These data and that of the phytochemicals implied that cattle were likely consuming some hay (November/December slaughter) and forage quality was likely lower at that time as indicated by lower amounts of hippurate and cinnamoyl glycline. Alkaloids may also interfere with the synthesis of b-vitamins in the rumen and alkaloids are found when alkaloid-containing plants “contaminate” hay or when more desirable plant species are lacking in the pasture (for example when more desirable grasses become dormant).

Slaughtering in late Spring/Early Summer and Early Fall could be ways to improve the vitamin content and phytochemical richness of meat. Incorporating more cool season grasses may be another opportunity to circumvent this. The interpretation by the research team is provided for educational purposes and should not be interpreted as diagnostic or treatment.

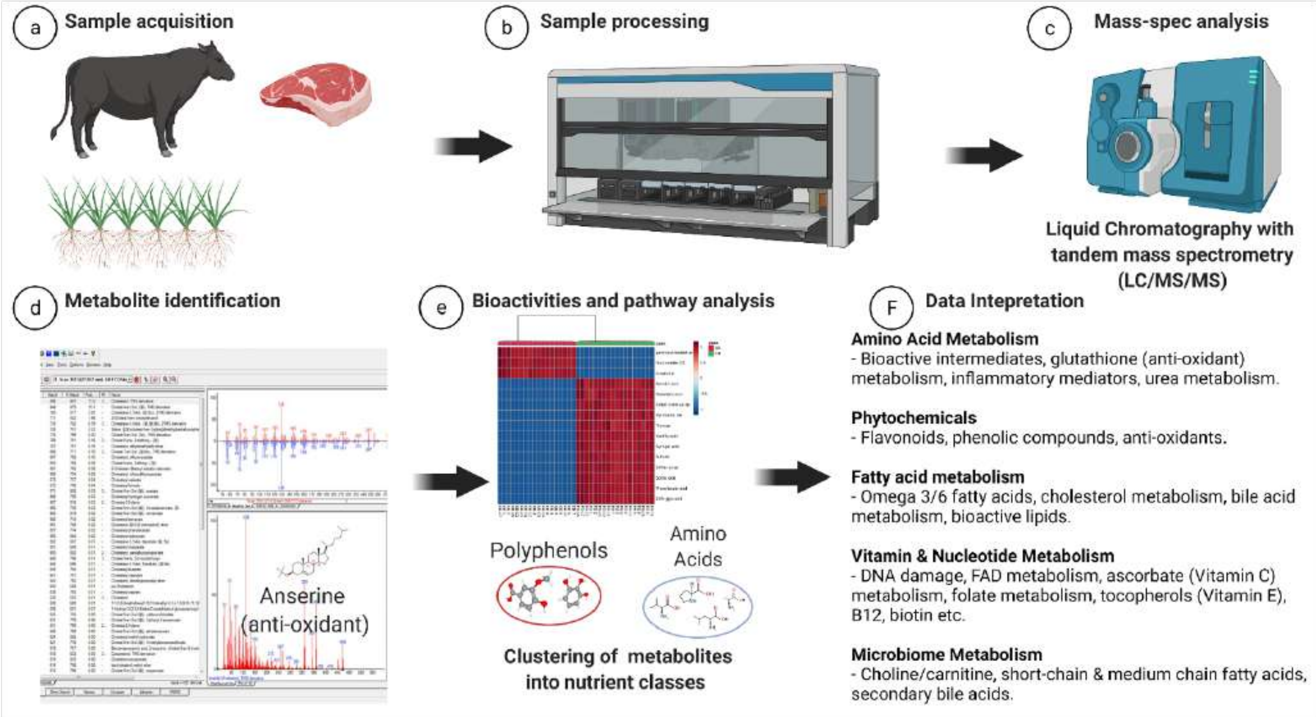
Objectives

The objective of our work was to use metabolomics approaches to provide an in-depth comparison of phytochemicals and biochemicals (>800 unique compounds) in submitted beef samples (see table below). Metabolomics is an analytical technique that allows researchers to simultaneously measure and compare large numbers of nutrients and metabolites present in biological samples. Metabolomics analysis of meat samples can simultaneously provide information on the metabolic health of the animal and the presence of potentially beneficial compounds for human consumption (nutrient density). A schematic representation of the sample information and the study flow is provided below.

Sample Information

Group ID	Treatment	Description
Sample Meats	Grass	Three Ribeye samples from three different beef cattle. Intensive rotational grazing. Pasture composition: Tall Fescue; Orchard Grass; Kentucky Blue Grass; Red Clover; White Clover; Broadleaf Plantain.
Grass-fed Average	Grass	Grass-fed Database Samples in Beef Nutrient Density Project
Grain-fed Average	Grain	Grain-fed Database Samples of Beef Nutrient Density Project

Sample Process



Schematic description of sample preparation and metabolomics analysis. **(a)** Samples were received and processed in the meat lab before further analysis. **(b)** Samples were processed in a wetlab using a series of extraction procedures, and **(c)** metabolomic analysis was conducted via LC-MS/MS. **(d)** Metabolites were identified using data extraction and peak identification software, and **(e)** data visualization tools including Metabolon Pathway Explorer, Reactome, and Metaboanalyst were used to inform **(f)** data interpretation including potential bioactivities and human health effects.

Results & Biological Interpretations

There is great consumer interest in whether different types of feeding and finishing strategies for animals produces differences in nutrition quality in meat, milk, and/or eggs. Data in the published literature supports the idea that pasture- and grain-based diets can result in differences in fatty acid profiles, vitamins, anti-oxidants, and animal health/welfare[2].

We identified >800 unique compounds in the beef samples, which can all be found in the provided excel sheet. These compounds included **phytochemicals/anti-oxidants, lipids (omega-3 fats), amino acids (proteins), vitamins and co-factors, peptides, mitochondrial metabolites, and carbohydrate molecules**. Below we will describe the main findings of our analysis focusing on the—to the best of our knowledge—most meaningful compounds in the samples. We describe what is known about these compounds in terms of health, and why we believe these compounds may be higher or lower based on the information provided. The data is contrasted with grass-fed and grain-fed beef samples within our database to inform findings.

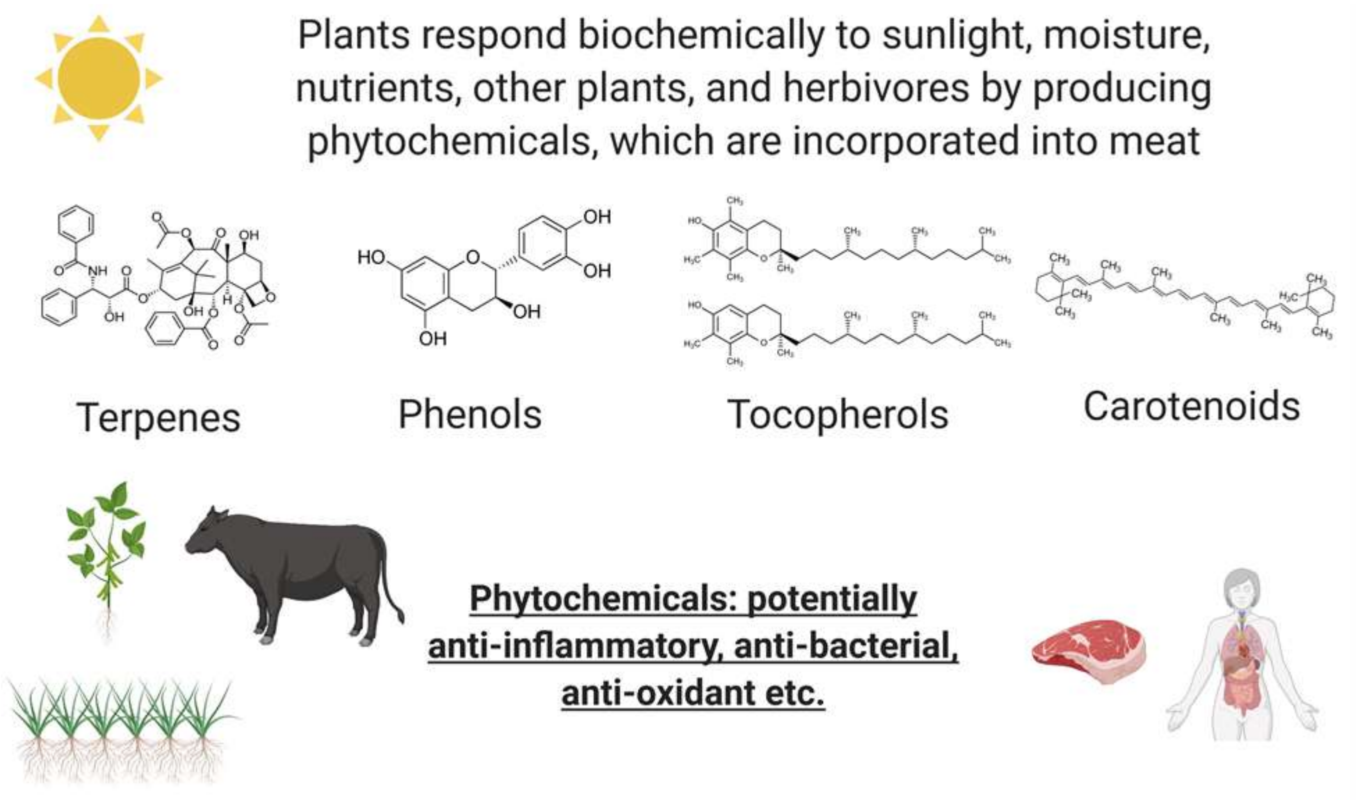
List of common compounds commonly mentioned in this report.

Nutrient	What it is	Potential impact on human health	Preferred Levels
Phyto chemicals	**Group of plant phenols with potential animal and human health benefits**	**Anti-inflammatory and anti-oxidant effects**	**Higher**
Hippurate	Phytochemical	↑ gut microbial diversity and ↓ risk of metabolic syndrome	Higher
Catechol-sulfate	Phytochemical: Downstream metabolite of hippurate	Inversely related to circulating cholesterol levels and anti-inflammatory	Higher
4-ethylphenyl-sulfate	Phytochemical: Downstream metabolite of hippurate	--	Higher
Phenol sulfate	Phytochemical/ polyphenol	--	Higher
Cinnamoyl-glycine	Phytochemical/ polyphenol	Anti-inflammatory, ↓ risk of Parkinson's disease and various cancers	Higher
N-methyl-pipecolate	Downstream metabolite of coumaric acid (phytochemical)	↓ oxidative stress and tumor activity in colorectal cancer models	Higher
Stachydrine	Phytochemical in alfalfa/ lucerne	Anti-oxidant, brain-protective, cardio-protective	Higher
Fatty acids			
Alpha-tocopherol	Vitamin E precursor	Anti-oxidant, cardio and brain-protective, ↑ eye sight	Higher
Vitamin C (ascorbate)	Water-soluble vitamin	Involved in tissue repair and enzyme activity of certain neurotransmitters	Higher
Vitamin B5 (pantothenic acid)	Water-soluble vitamin	It helps produce energy by breaking down fats and carbohydrates. It also promotes healthy eyes, skin and liver.	Higher
Vitamin B6 (pyridoxine)	Water-soluble vitamin	Essential anti-oxidant for brain health and immune system	Higher
Vitamin B3 (Niacin)	Water-soluble vitamin	Healthy nervous system, skin, and digestive system	Higher
Choline	Essential Nutrient	Brain, muscle, liver function	Higher

Fatty acids			
EPA	Part of the omega-3 family	Anti-inflammatory, anti-oxidant, ↓ associative risk of heart disease, and liver disease, ↑ brain function	Higher
DHA	Part of the omega-3 family	Anti-inflammatory, anti-oxidant, ↓ associative risk of heart disease, cancer, and liver disease. ↑ brain function	Higher
Linolenic acid	Part of the omega-3 family	↓ risk of CVD, ↑ brain health	Higher
Long chain saturated fats		↓ risk of diabetes and heart disease	Higher
Caprate	Part of the long chain saturated fat family	Anti-bacterial, anti-fungal, anti-inflammatory	Higher
Lauric Acid	Part of the long chain saturated fat family	Aids in recovery of viral infections	Higher
Triglycerides	--	↑ heart health	Lower
Energy metabolites			
Long chain acyl carnitines	Important for transport of nutrients to mitochondria	↑ heart health	Higher
Citrate	Mitochondrial/energy metabolite	More "athletic" animal, likely grass-fed	Higher
Succinate	Mitochondrial/energy metabolite	More "athletic" animal, likely grass-fed	Higher
Malate	Mitochondrial/energy metabolite	More "athletic" animal, likely grass-fed	Higher
Amino acid metabolites			
Homocysteine	Amino acid	↓ risk of heart disease	Lower
Carnitine	Antioxidant in animals	Helps the body turn fat into energy, important for heart and brain function	Higher
Anserine	Derivative of carnosine	Anti-oxidant, important for brain function	HIgher

*This list is non-exhaustive and represents an overview of potential health benefits based on associative data, experimental animal data, and in vitro-based models published in the scientific literature. How consumption of these compounds through meat impact human health is not always known or a given. The interpretation by the research team is provided for educational purposes and should not be interpreted as diagnostic or used for disease treatment. Nor does the data mean that eating meat from different production systems will, as a result, increase or decrease disease risk.

Phytochemicals



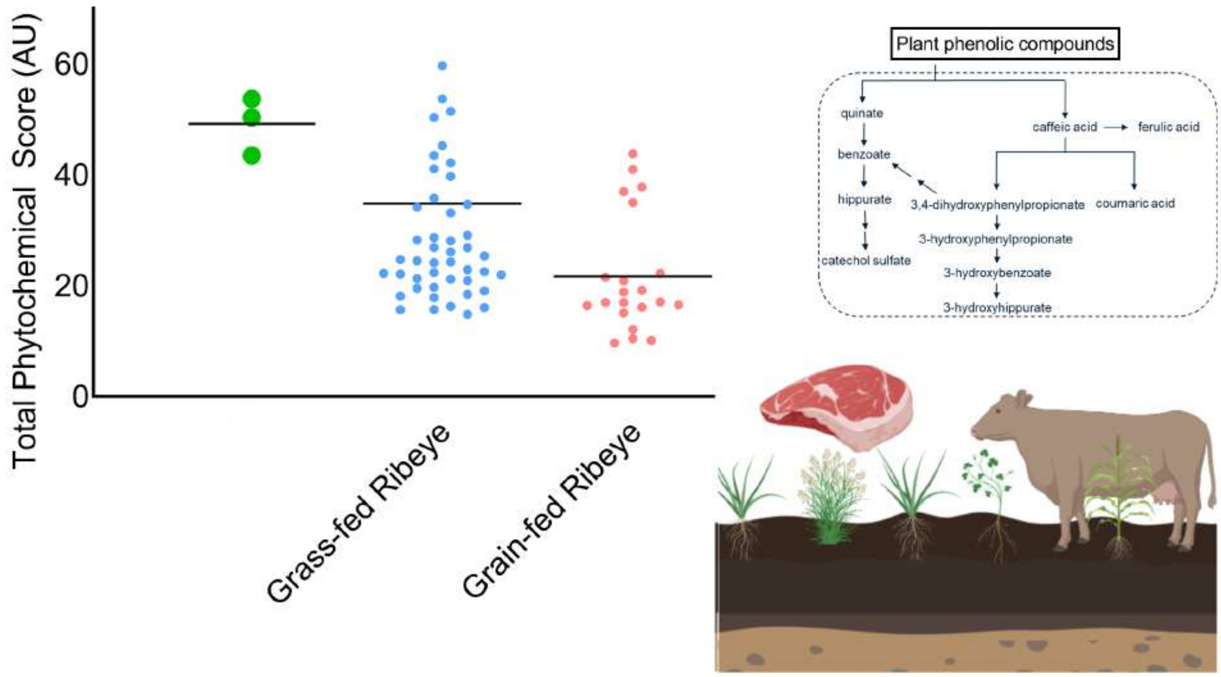
Grasses and forbs are rich in antioxidant compounds and vitamins, including tocopherols, carotenoids, terpenes, and polyphenols. **Phytochemicals** are plant-derived bioactive compounds that have been studied for their role in both animal[2] and human health[3]. Their roles include (1) having anti-inflammatory/antioxidant effects; (2) scavenging reactive or toxic chemicals; (3) enhancing the absorption and or stability of essential nutrients; and (4) acting as selective growth factors for beneficial gut bacteria. They may play roles in preventing and managing chronic diseases including cancers[4], heart disease[5], diabetes[6], high blood pressure[7], inflammation[8], microbial, viral and parasitic infections[9], and neurological disease[10]; though further research in humans is certainly needed, especially as it relates to obtaining these compounds from meat.

Nonetheless, having more phytochemicals in meat could be considered beneficial for animal health and may improve the healthfulness of meat[11], which is currently studied in randomized controlled trials. Phytochemicals obtained from the forage/feed are metabolized by microbial populations in the rumen and/or are further metabolized by the liver. In the meat, they may be detectable as their parent compound (the same intact compound as found in the plant), but also as metabolized compounds (downstream metabolites; see figure below for examples of downstream metabolites).

Total Phytochemicals

Name: Total phytochemical score

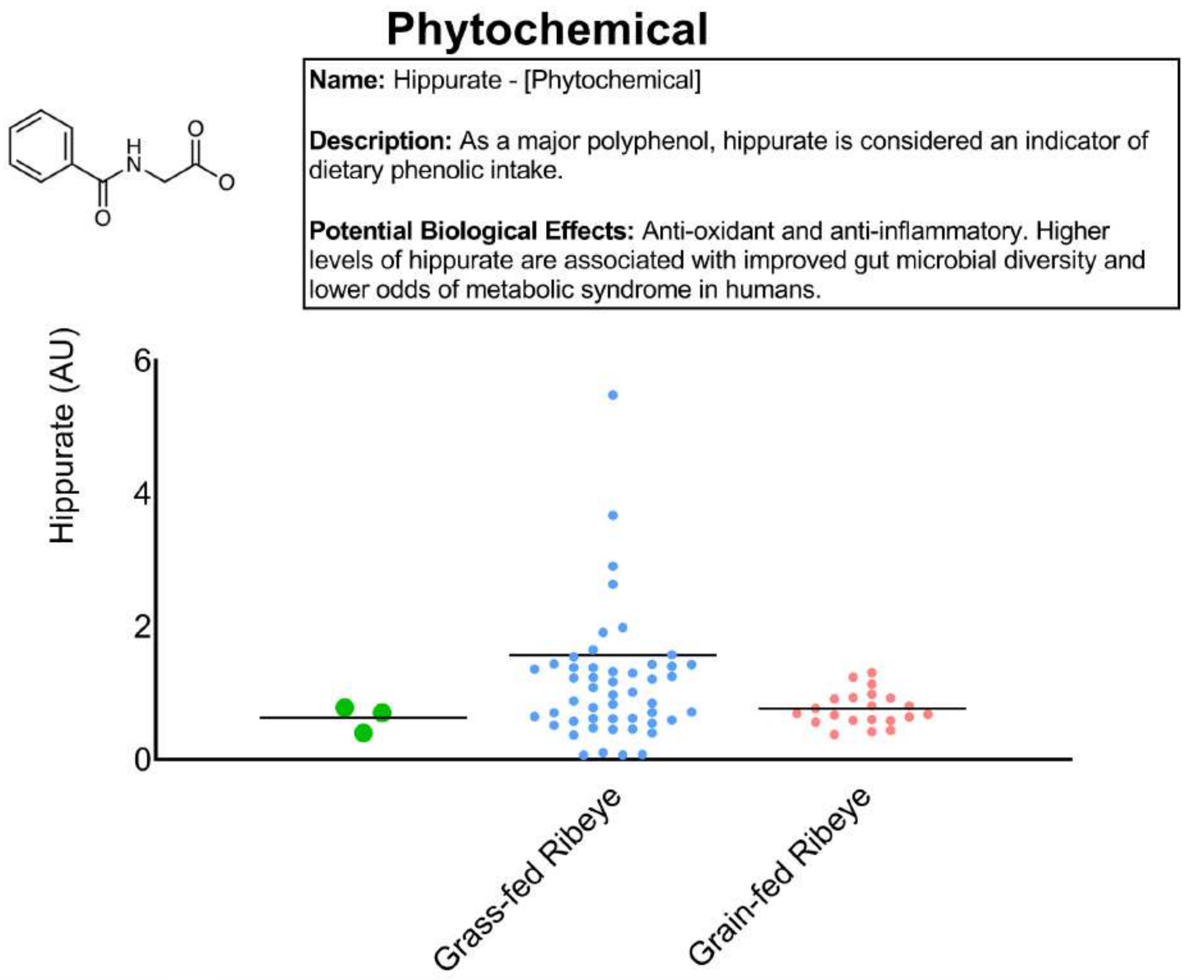
Description: Phytochemicals are compounds that are produced by plants ("phyto" means "plant") and include terpenoids, flavonoids, flavanols, and other (poly)phenols. These compounds are believed to may have anti-oxidant and anti-inflammatory effects and lower oxidative stress. Oxidative stress has been implicated in the development of neurodegenerative disease, cardiovascular disease, and diabetes.



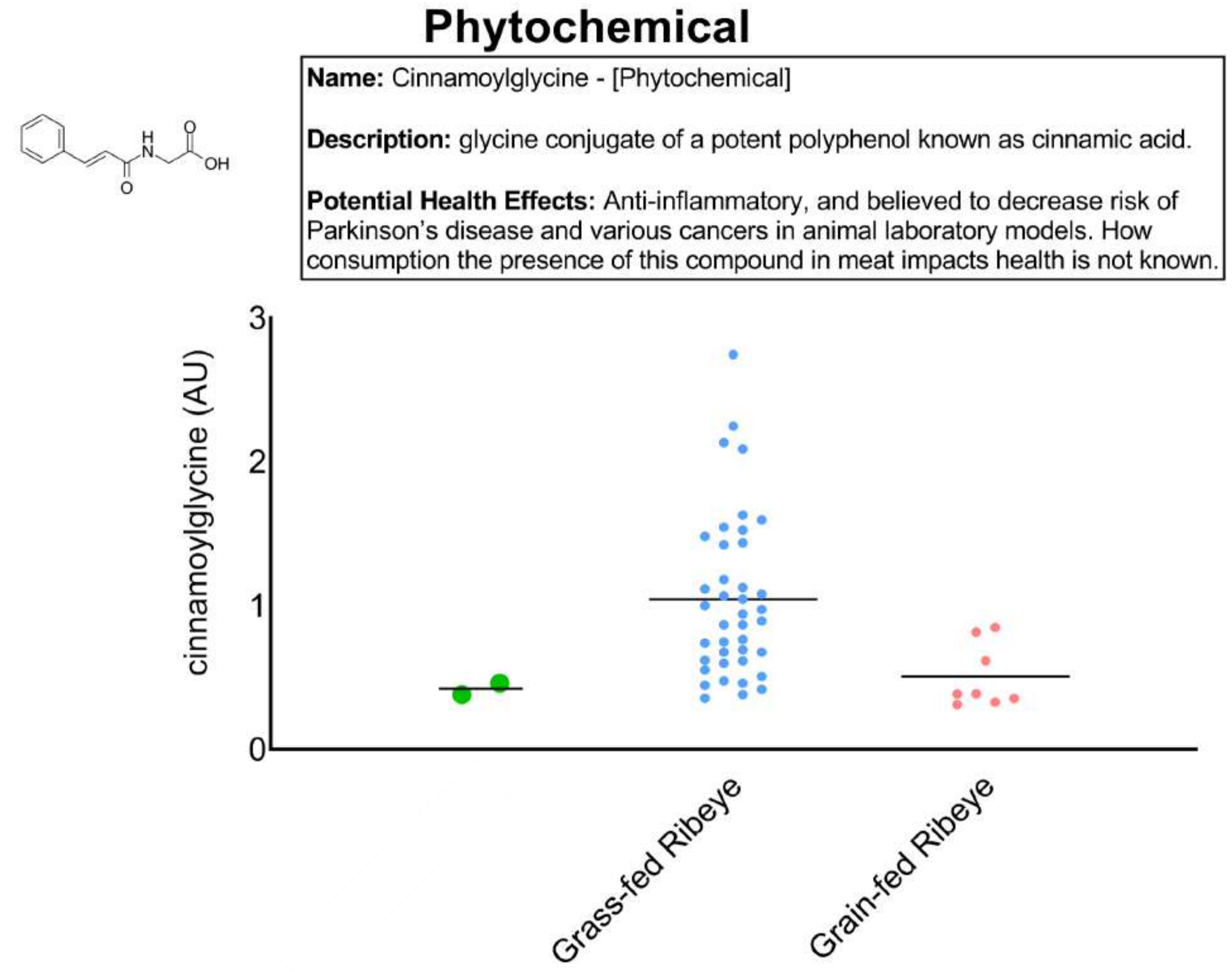
The total phytochemical score is the sum of phytochemicals measured in the samples, which are summarized in the excel file and below. Consistent with findings in the literature and our analysis, we typically find that animals grazing a more biodiverse pastures (>5-10 plants) concentrate more phytochemicals than when animals are grazing more monoculture pastures and low-quality grasses. However, even in the case of feedlot-finishing systems there are opportunities to increase the phytochemicals/antioxidants in meat as certainly there is considerable variation in such systems as shown above. Feeding a greater proportion of phytochemically-rich hay/alfalfa does result in higher amounts of phytochemicals in meat and milk. Thus, feeding some phytochemically-rich agriwaste (e.g., almond hulls, potato peels, grape byproducts, and/or other fruit/vegetable byproducts), if accessible, can be beneficial.

We typically also see an effect of **seasonality**. Samples from the Spring/early Summer and early Fall contain different phytochemicals than when animals are on conserved forages. Drought in the summer may also reduce the phytochemical richness as forage becomes wilted. Finally, when pastures are **overgrazed/continuously grazed**, we also typically see lower amounts of phytochemicals in meat as grasses can become of low-quality as a result. Incorporating a diverse seeding mixture, re-establishing native vegetation, planting cover crops, and/or ensuring adequate rest periods are all ways to potentially increase the phytochemical richness of the meat in grazing systems. The implementation and feasibility of this should be tailored towards the individual farm and based on the local ecosystem.

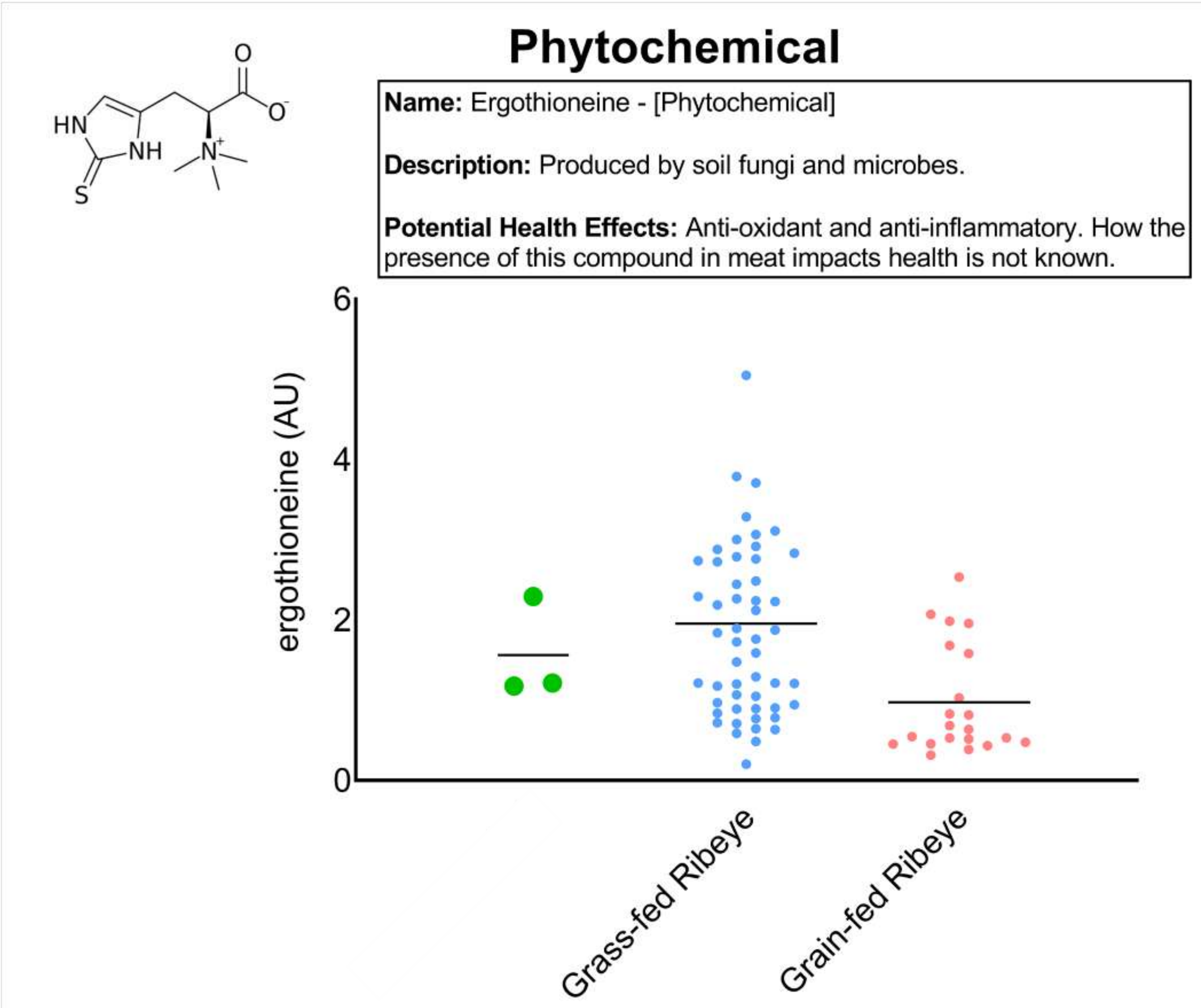
Hippurate, a major polyphenol, is considered a strong indicator of dietary phenolic intake[12]. Higher levels of **hippurate** are associated with improved gut microbial diversity and **lower odds of metabolic syndrome** in humans[13]. **Catechol sulfate**, which is a downstream metabolite of hippurate, is inversely associated with circulating cholesterol levels and indicated to have anti-inflammatory effects in humans. We find these compounds to be good indicators of the phytochemical richness of samples, with this compound being especially high when the animals are on fresh forages.



Cinnamoylglycine is the glycine conjugate of a potent polyphenol known as cinnamic acid. Cinnamic acid and its metabolites may have anti-inflammatory effects[14] and higher intakes are linked to a number of health benefits in animal models including reduced risk of Parkinson's disease[15] and reduced risk of various cancers[16,17].

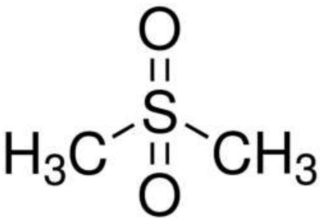


Ergothioneine, and its oxidative metabolite histidine betaine (hercynine), are betaine amino acids with potential antioxidant activities and have been proposed as compounds that can lower oxidative stress. Oxidative stress is at the root of many metabolic diseases. Ergothioneine is synthesized by a variety of microbes, especially fungi) and actinobacteria, but is not synthesized by plants and animals who acquire it via the soil and their diet, respectively. These compounds are produced by Actinomycetota, Cyanobacteria, and certain fungi in the rhizosphere. Higher amounts of this compound may indicate good soil health.



Dimethyl sulfone is a naturally occurring organosulfur compound utilized in complementary and alternative medicine and is studied for its antioxidant capabilities and its anti-inflammatory effects, as well as in cancer medicine[18]. Dimethyl sulfone is often found in native plants of the horstetail family. While this compound can have health benefits, there is some indication it can be toxic to livestock at substantial amounts. Horsetail is generally unpalatable due to its high silicate content and studies have shown grazing cattle to avoid marsh horsetail in the presence of other palatable forage, but may occasionally ingest this depending on the available forages[19]. If amounts are too high, ruminants can develop thiamine deficiency.

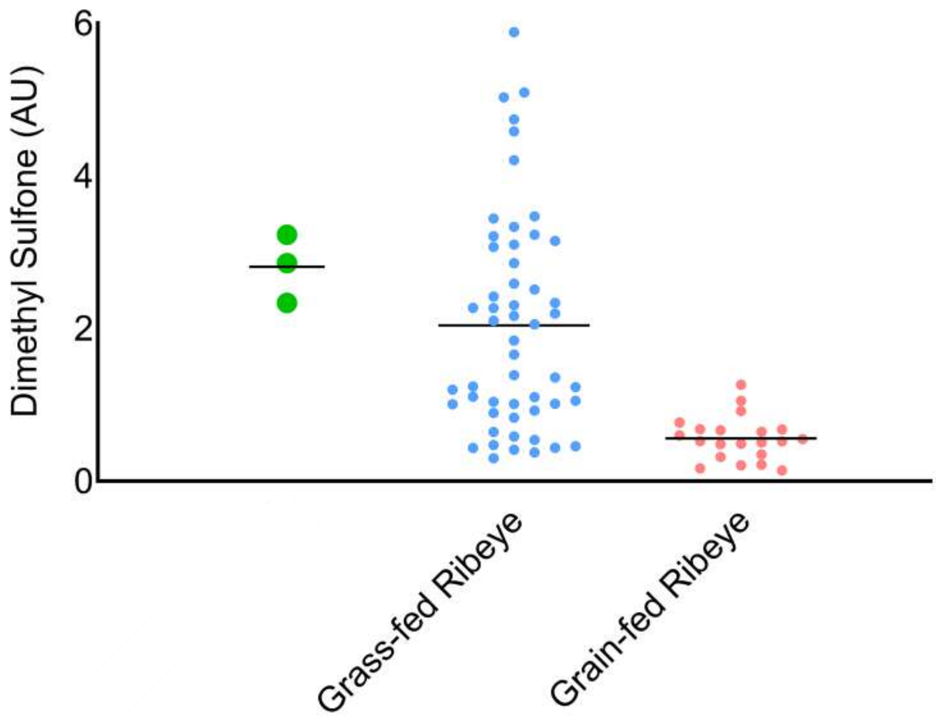
Phytochemical



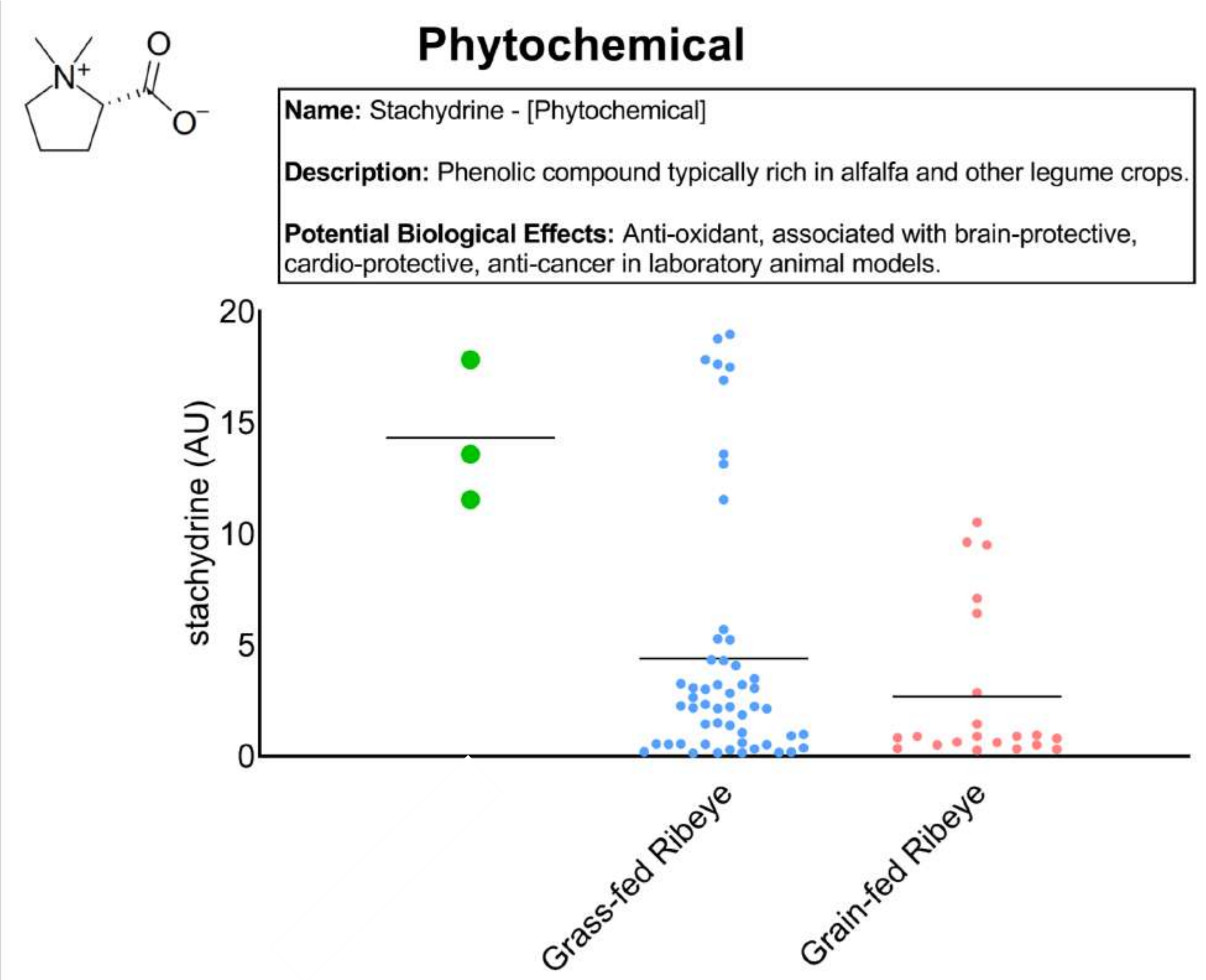
Name: Dimethyl Sulfone - [Phytochemical]

Description: Dimethyl sulfone is an alkaloid found in native plants of the horsetail family. In substantial amounts this plant could be toxic to livestock.

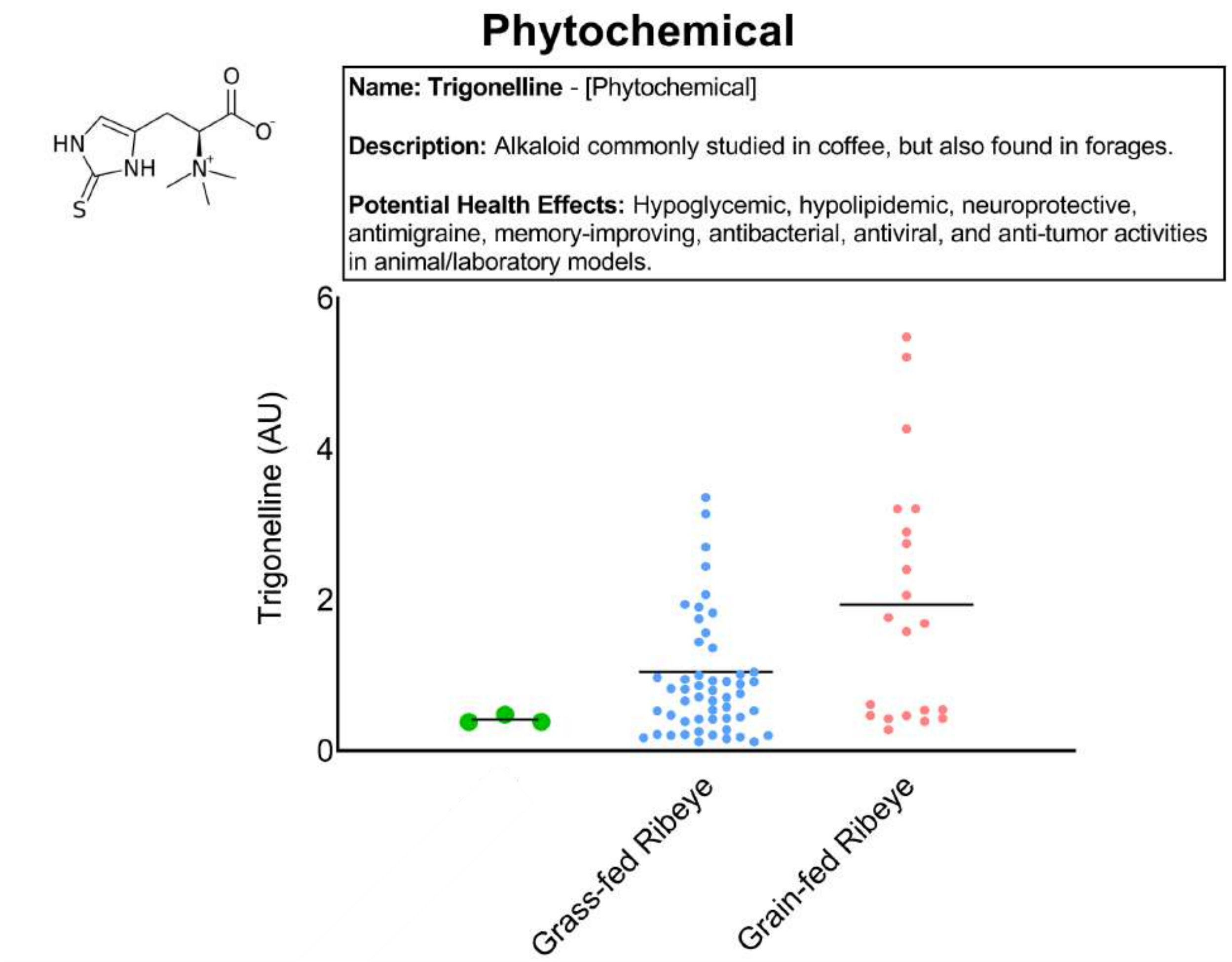
Potential Health Effects: Anti-oxidant and anti-inflammatory. How the presence of this compound in meat impacts health is not known.



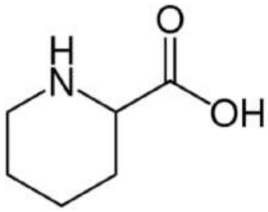
Stachydrine and **homostachydrine** are two compounds highly concentrated in plants from the Stachys genus. Stachys is one of the largest genera in the flowering plant family Lamiaceae, and includes plants such as alfalfa[20]. Animal and in vitro models suggest that **stachydrine** has anti-oxidant properties and may have brain-protective[21,22], cardio-protective[23], and anti-cancer effects[24]. Typically, higher amounts of these compounds suggests that animals have been fed conserved forages/hay. This compound is especially rich in animals fed alfalfa-hay.

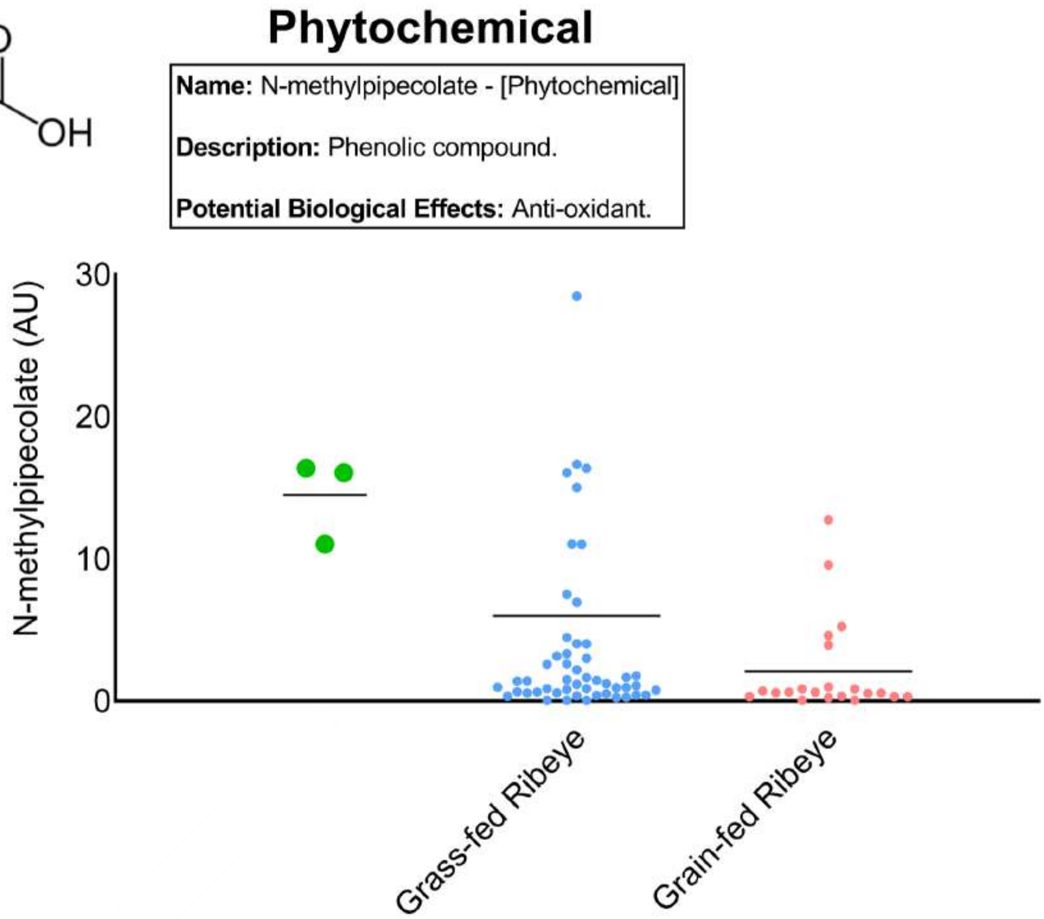


Trigonelline has been isolated from fenugreek seeds (Trigonella foenum-graecum, hence the name), peas, hemp, oats, Stachys species (such as alfalfa), dahlia, Strophanthus species,and Dichapetalum cymosum. Trigonelline is also found in coffee and has been studied especially regarding diabetes and central nervous system disease. Trigonelline has hypoglycemic, hypolipidemic, neuroprotective, antimigraine, sedative, memory-improving, antibacterial, antiviral, and anti-tumor activities, and it has been shown to reduce diabetic auditory neuropathy and platelet aggregation. It acts by affecting β cell regeneration, insulin secretion, activities of enzymes related to glucose metabolism, reactive oxygen species, axonal extension, and neuron excitability. However, further study of trigonelline's pharmacological activities and exact mechanism is warranted, along with application of this knowledge to human health.



N-Methylpipecolate is derived from pipecolic acid, which is a lysine-derived compound found in various organisms, including bacteria, fungi, animals, and plants. Pipecolic acid preconditions plants for optimal production of the phenolic defensive signal salicylic acid (SA). This compound is believed to have potential anti-oxidant effects. We find this compound to be especially enriched when animals are fed hay.





Fatty Acids

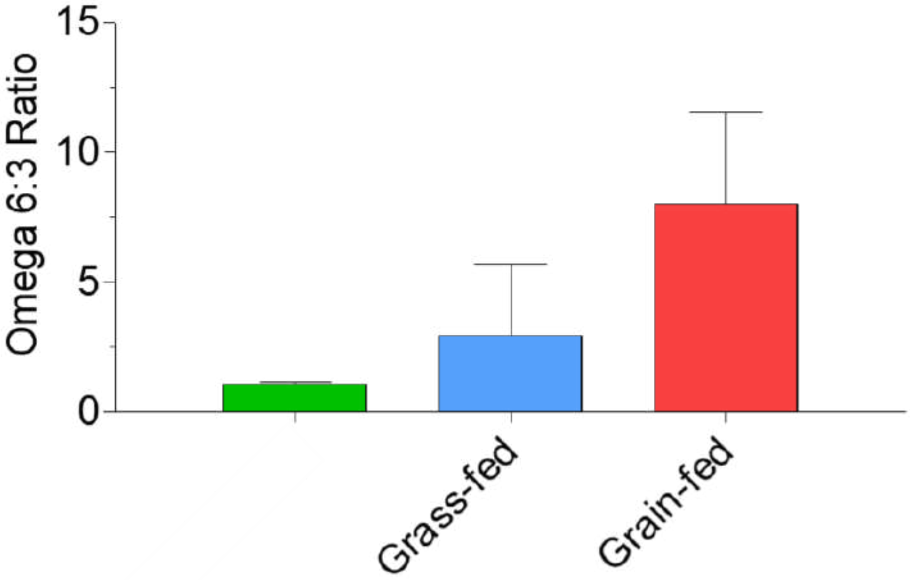
Health claims associated with grass-fed meat often focus on the ratio between **omega-6 and omega-3** PUFAs[11]. The omega-3 fatty acids have well-known health benefits including anti-inflammatory and antioxidant properties. They may help lower the risk of getting heart disease, cancer, and liver diseases and could help improve brain function[25]. A lower ratio is typically considered more favorable for human health.

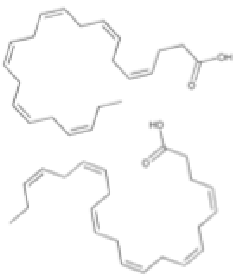
The omega-6 to-3 ratio is the direct result of the amounts of the very long-chain polyunsaturated omega-3 fatty acids, **docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and alpha linolenic acid (ALA)**. These omega-3 fatty acids can have important health benefits throughout life, and higher intakes are associated with reduced risk of heart disease and improved brain function[25]. While ruminant meat is sometimes not considered a meaningful dietary source of omega-3 fatty acids, several randomized controlled trials have reported that consumption of meat from ruminants with increased omega-3 levels can increase circulating levels of EPA, DPA, and/or DHA in consumers[26-29].

Name: Omega 6:3 Ratio - [Fatty Acid]

Description: The ratio of omega-6 fatty acids divided by omega-3 fatty acids.

Potential Health Effects: A lower omega 6:3 ratio is typically considered beneficial. Lower levels means a higher abudance of omega-3 fatty acids. Randomized controlled trials suggest that beef with a low omega 6:3 ratio can increase omega-3 blood levels in consumers, which is generally considered beneficial for human heath.

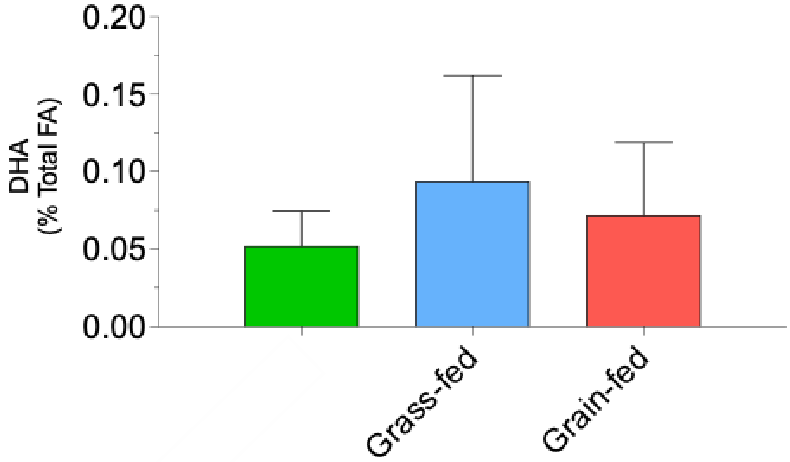




Name: Docosahexaenoic acid (DHA) - [Fatty Acid]

Description: DHA is an omega-3 fatty acid that is a primary structural component of the human brain, skin, and retina. Typically DHA is higher in grass-fed beef due to higher intakes of its plant precursor ALA.

Potential Health Effects: The omega-3 fatty acids DHA and EPA have well-known health benefits including anti-inflammatory and antioxidant properties. They may help lower the risk of getting heart disease, cancer, and liver diseases and could help improve brain function. Higher levels are considered beneficial.

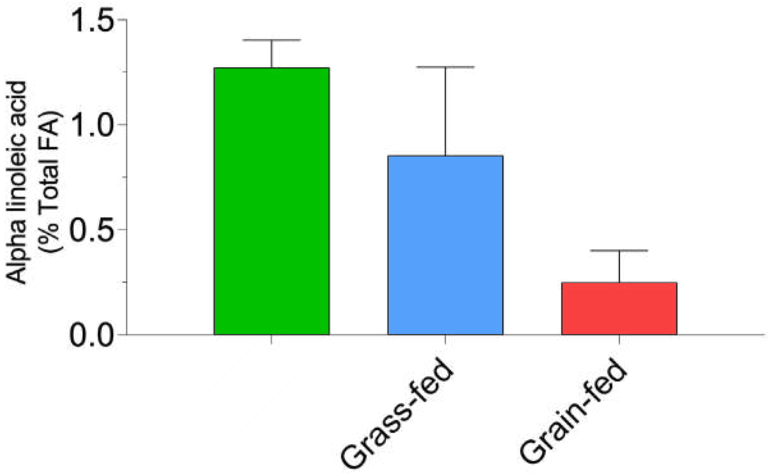


ALA is the plant precursor to longer-chain omega-3s such as DHA and EPA and may have distinct health benefits from DHA and EPA. ALA is typically rich in fresh forages, but lower in grains. **Linoleic acid** is the major omega-6 found in meat and typically increases with grain-feeding and or hay/silage feeding.

Name: Alpha Linolenic Acid - [Fatty Acid]

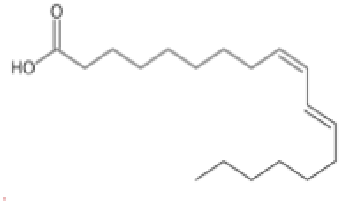
Description: Alpha linolenic acid (ALA), is an essential fatty acid that belongs to the omega-3 group. It is typically rich in fresh forages. It is also a precursor to to docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are omega-3 fatty acids with well-known health benefits.

Potential Health Effects: ALA is associated with reduced risk of cardiovascular disease and improved brain health and may have distinct benefits from DHA and EPA.



Population-based studies[30-33] and randomized controlled trials[34] link higher intakes of palmitate to increased risk of cardiovascular disease relative to other (longer) chain saturated fatty acids such as stearic acid and arachidic acid; with the latter often being associated with a reduced risk of cardiovascular disease[33]. Like the long-chain omega-3s, long-chain saturated fatty acids also become enriched when animals are on forage-based diets and are typically reduced when animals are consuming grain-based concentrates.

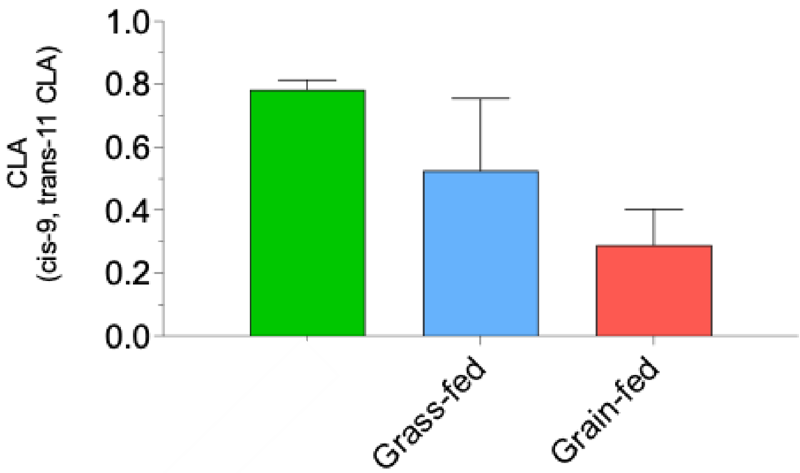
Meat from grass-fed ruminants is typically a rich source of **conjugated linoleic acid (CLA)**. CLA is predominantly studied for its anti-cancer and fat loss effects and show benefit in some population-based studies[35,36]. Pasture management will play an important role in forage fatty acid composition[37]. Animals grazing fresh grass have higher levels of omega-3 fatty acids and CLA in their meat or milk, when compared to those consuming conserved forages such as hay/silage and/or wilted grasses[37]. This is explained by the loss of omega-3 precursors during silage/hay making, as well as when grasses are wilting or overgrazed.

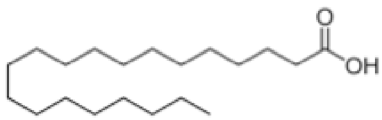


Name: Conjugated Linoleic Acid (CLA) - [Fatty Acid]

Description: Conjugated linoleic acid (CLA) is a fatty acid that is typically rich in meat from animals that are fed fresh forages.

Potential Health Effects: CLA is predominantly studied for its anti-cancer and fat loss effects and is associated with benefits in some but not all population-based studies. Higher levels are considered beneficial.

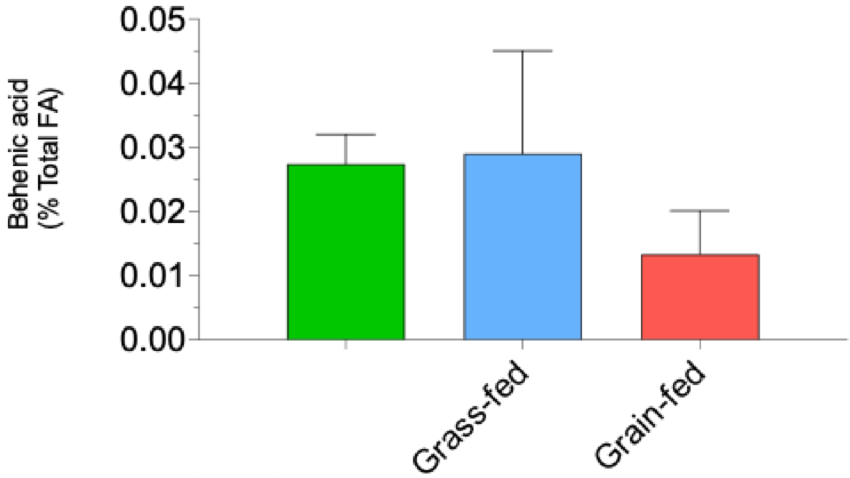




Name: Behenic Acid - [Fatty Acid]

Description: A very long-chain saturated fatty acid.

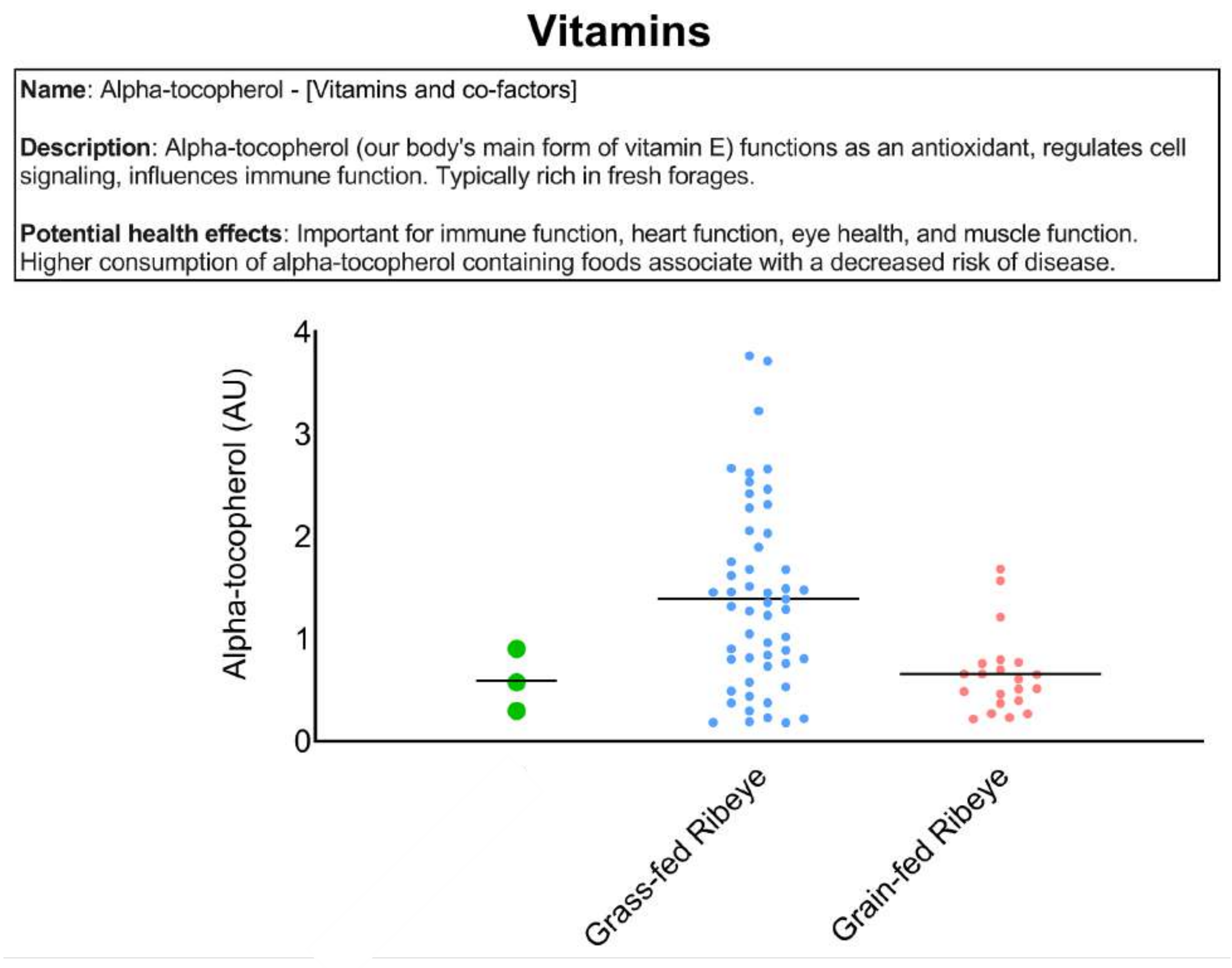
Potential Health Effects: Higher circulating concentrations of long-chain saturated fatty acids such as arachidate and behenate are associated with a decreased risk of diabetes and cardiovascular disease.



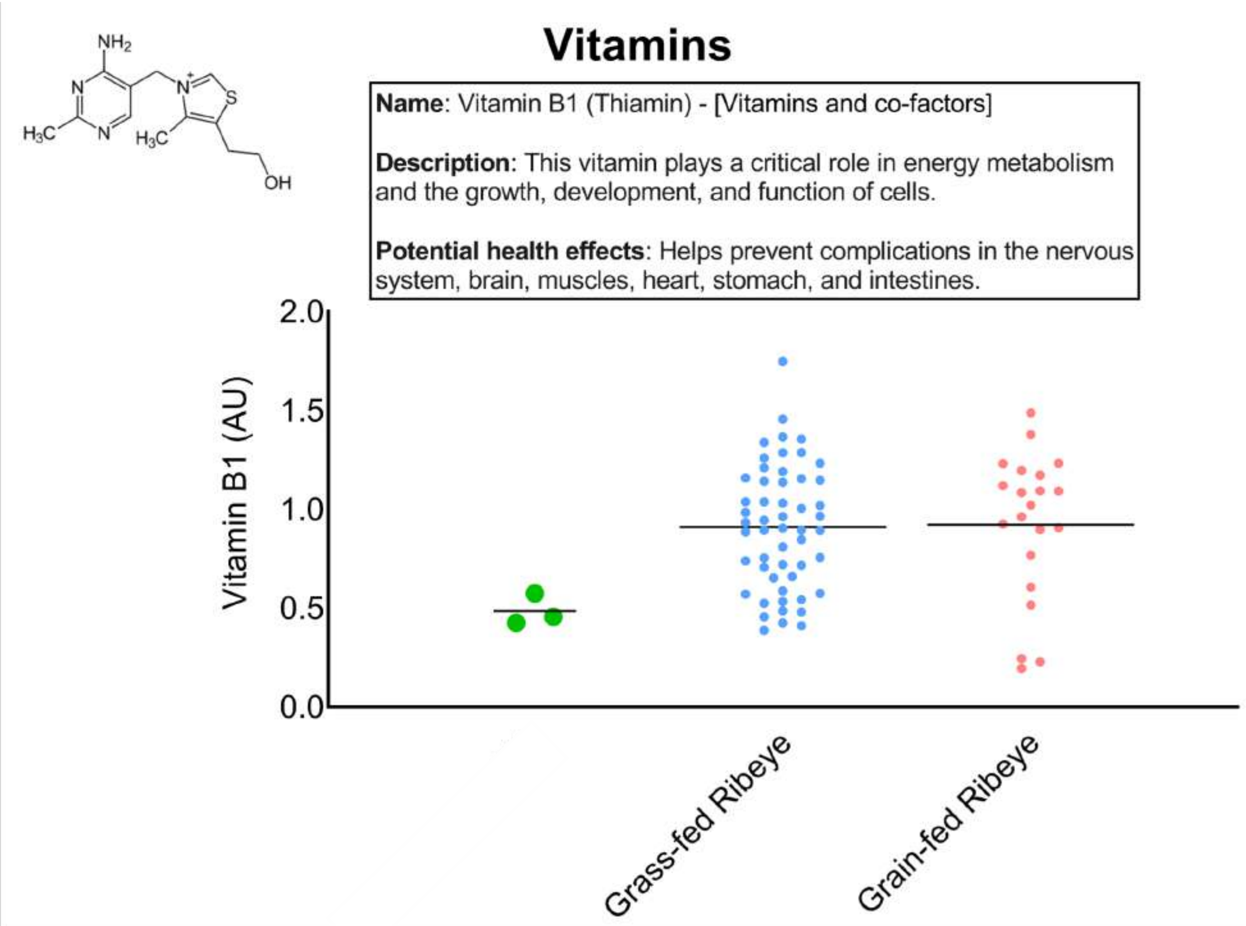
Vitamin Metabolites

Meat is a common source of **B-vitamins**, and **alpha-Tocopherol (Vitamin E).**

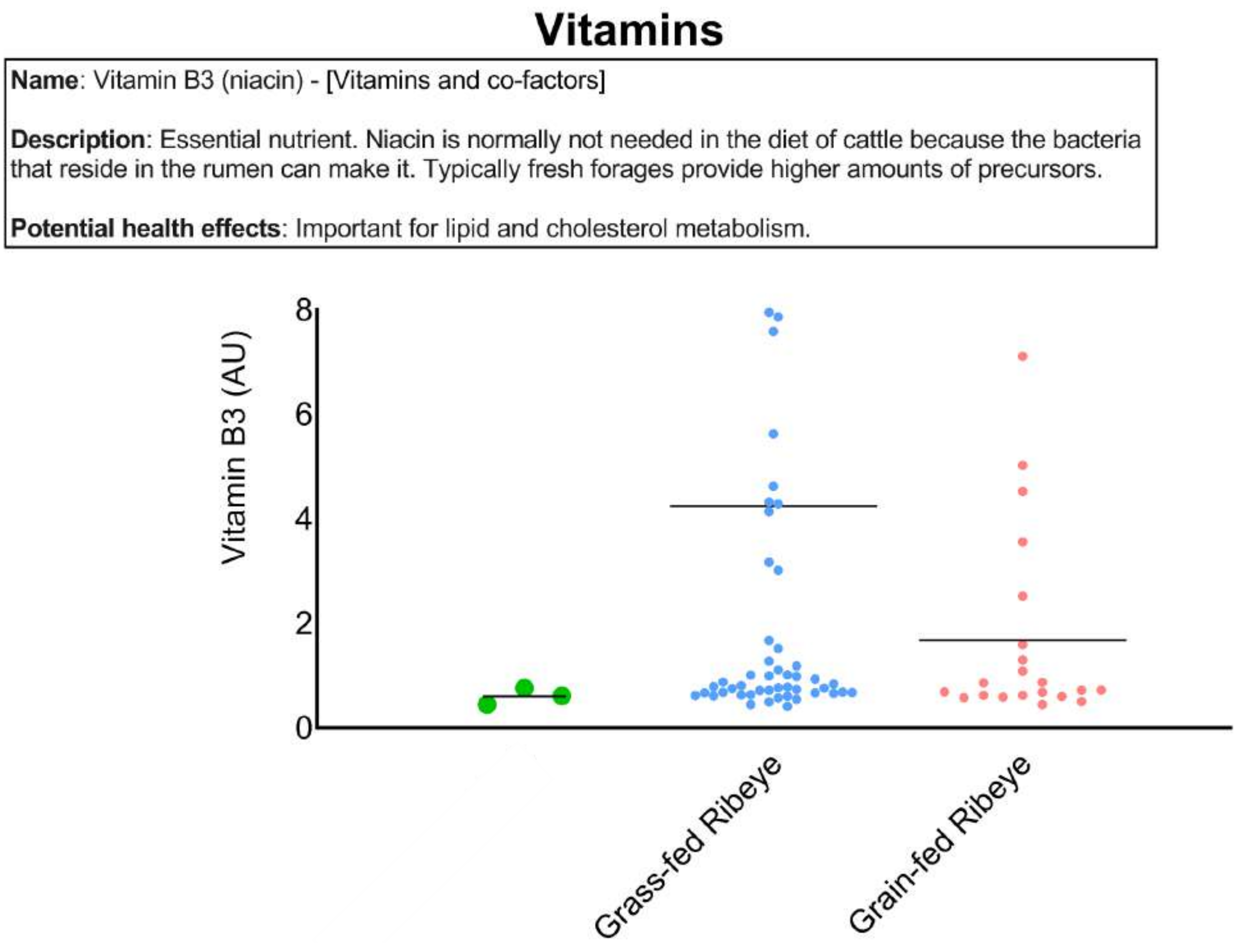
Alpha-tocopherol is a vitamin E precursor best known for its anti-oxidative effects. Tocopherols are associated with protection against cardiovascular disease[37], certain cancers[38,39], brain function decline[40], and reduced eye-sight[41]. Tocopherol is particularly rich in plantain and birdsfood trefoil forages. Typically, alpha-tocopherol is highest in fresh forages, reduced in conserved forages/low quality grasses, and lowest in grain-based concentrates. Alpha-tocopherol is the most prevalent tocopherol in mammalian tissue and exhibits higher biological activity and anti-inflammatory properties than gamma- and beta- tocopherol[42]; however, gamma-tocopherol has health benefits that distinguish it from alpha-tocopherol. Gamma tocopherol is typically richer in grain-fed meat, which may be the result of the relatively high presence of beta-/gamma-tocopherol in alfalfa [65] and corn [66].



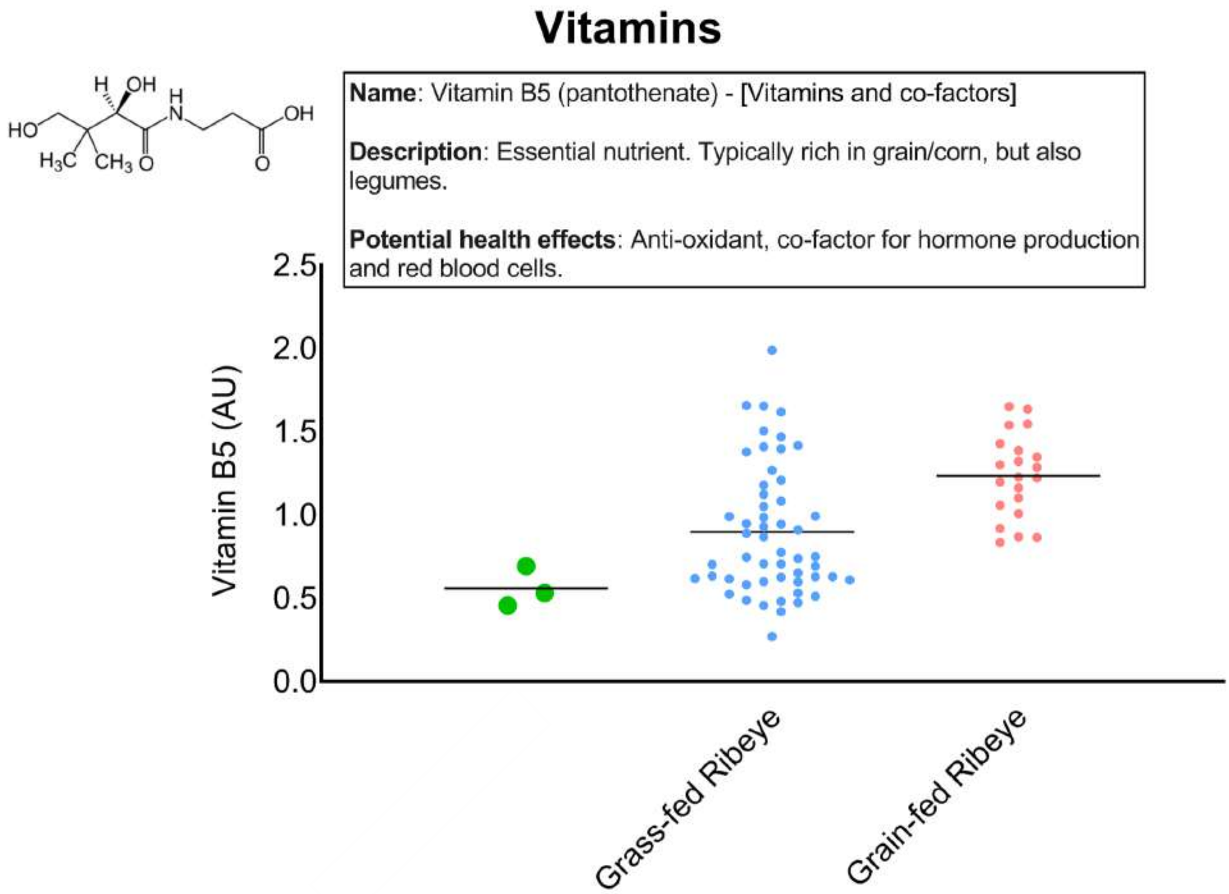
Vitamin B1 is normally produced by bacteria in the rumen on well-balanced roughage diets. Typically, thiamine deficiency can be caused by reduced production by rumen microbes or factors that affect the action of thiamine. Thiamine deficiency is usually caused by plant thiaminases that destroy thiamine or hinder the thiamine function. Horsetail and bracken fern contains significant amount of thiaminases and can lead to lower thiamine level in animals. If dimethyl sulfone levels are up (see phytochemicals) this could be an indication that alkaloid intake is high and may interfere with Vitamin B1 synthesis.



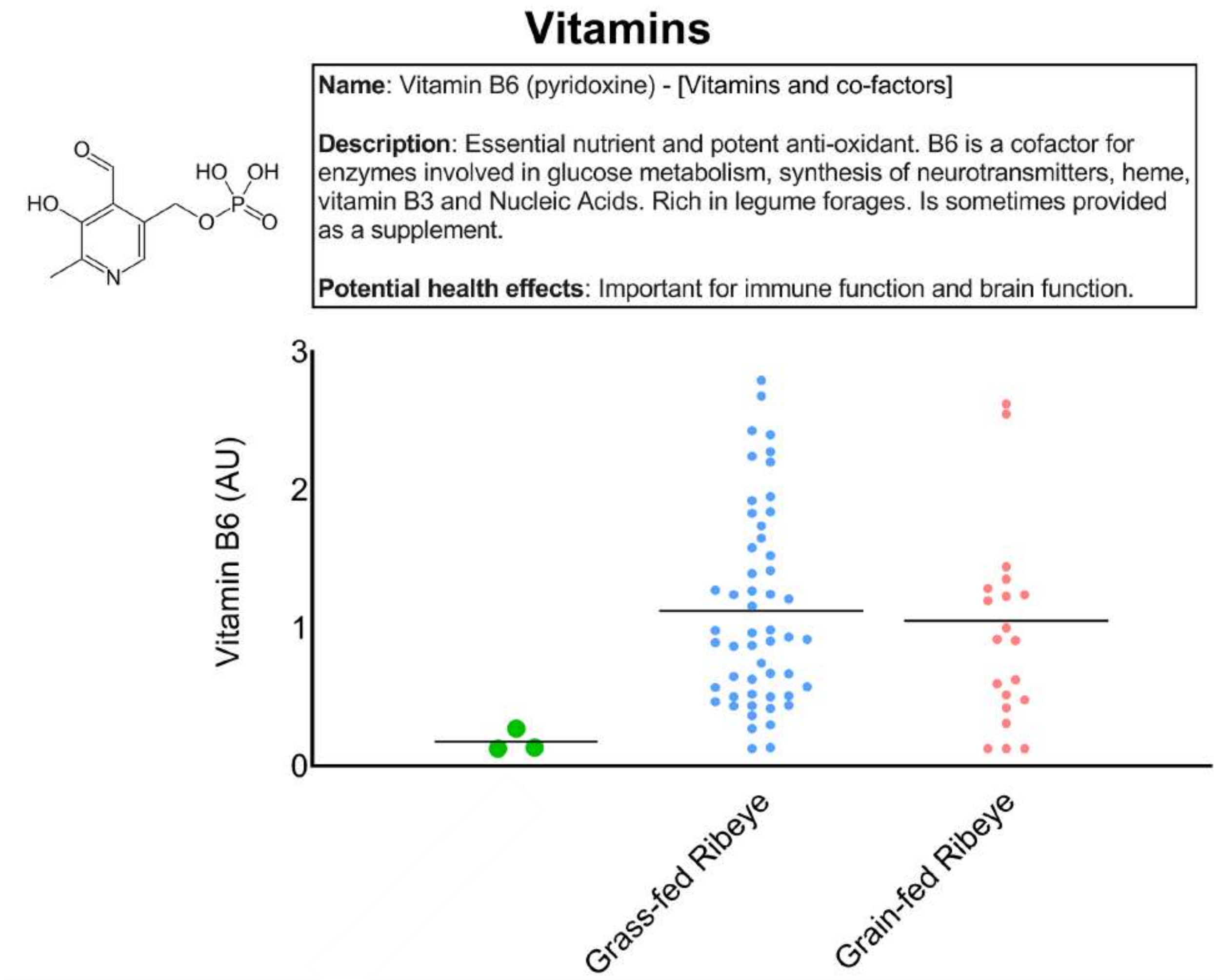
Niacin (Vitamin B3) is used by the body to turn food into energy. This vitamin help promote a healthy nervous system, digestive system, and skin. Higher amounts in meat may be considered beneficial. Niacin composition of meat is largely dependent on diet composition of cattle, with fresh forages being a good precursor[43].



Pantothenate (Vitamin B5) is an essential nutrient that has anti-oxidant roles, and acts as a co-factor for hormone production and red blood cells. Vitamin B5 (pantothenic acid) is critical for animal and human health because it is involved in a vast number of chemical reactions within the body. Typically, we find this vitamin to be higher in grain-fed beef samples as corn, soybean-meal, and alfalfa are a rich source of this nutrient. Grazing animals can manufacture their own vitamin B5 from the microbiomes in their rumen when provided with well-grown green forages, especially alfalfa, ryegrass, and legumes (including peas)[44].

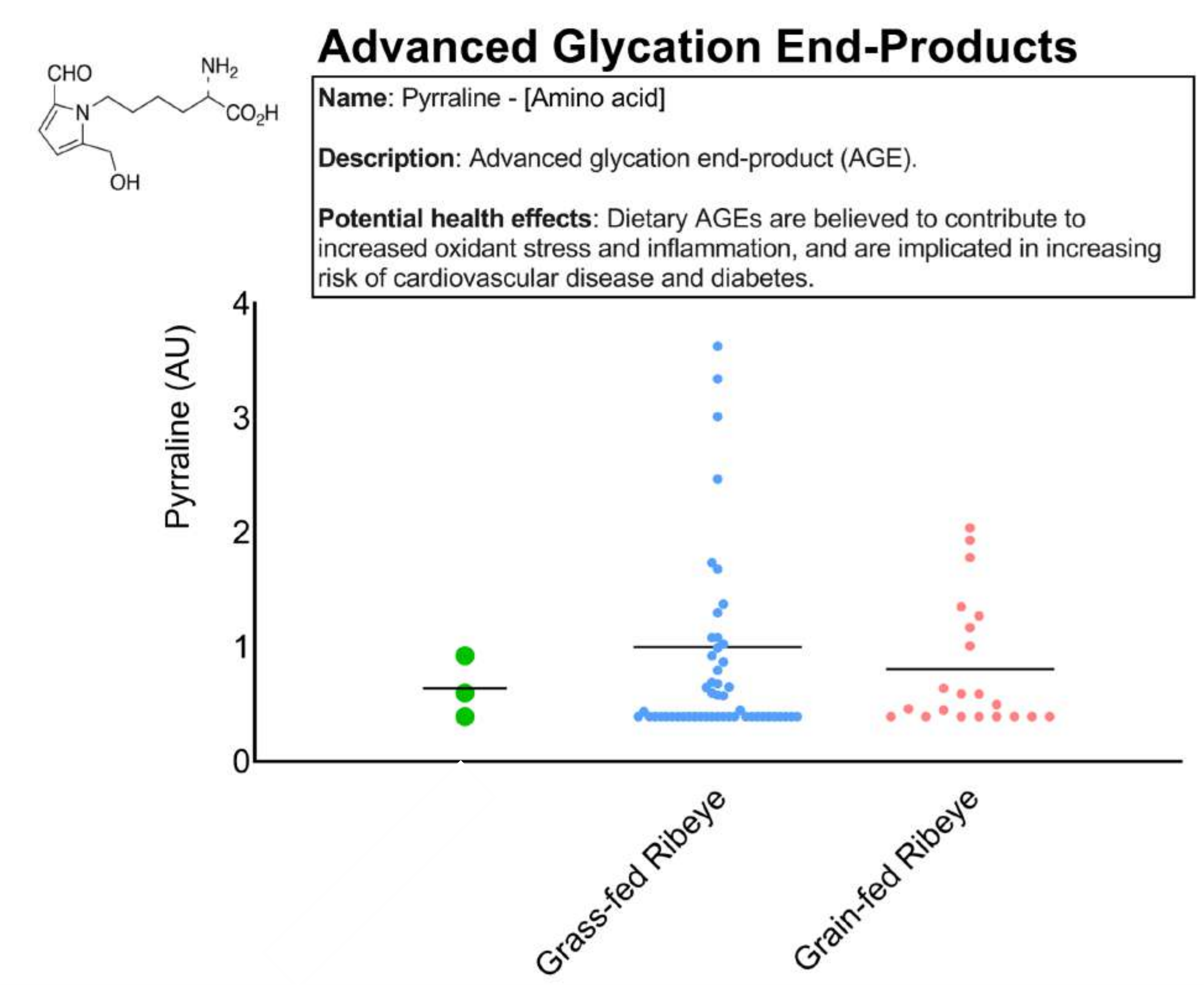


Pyrodixine (Vitamin B6) is needed for the proper function of sugars, fats, and proteins in the body. It's also necessary for the development of the brain, nerves, skin, and many other parts of the body. Forage-based diets improve various b-vitamins[45,46]. B3 and B6 are especially in leguminous forages (barley, triticale), clover, and maize and alfalfa silage in the case of feedstuff. Incorporating clover, oats, peas, triticale and other leguminous forages could be a way to increase these amounts in beef.

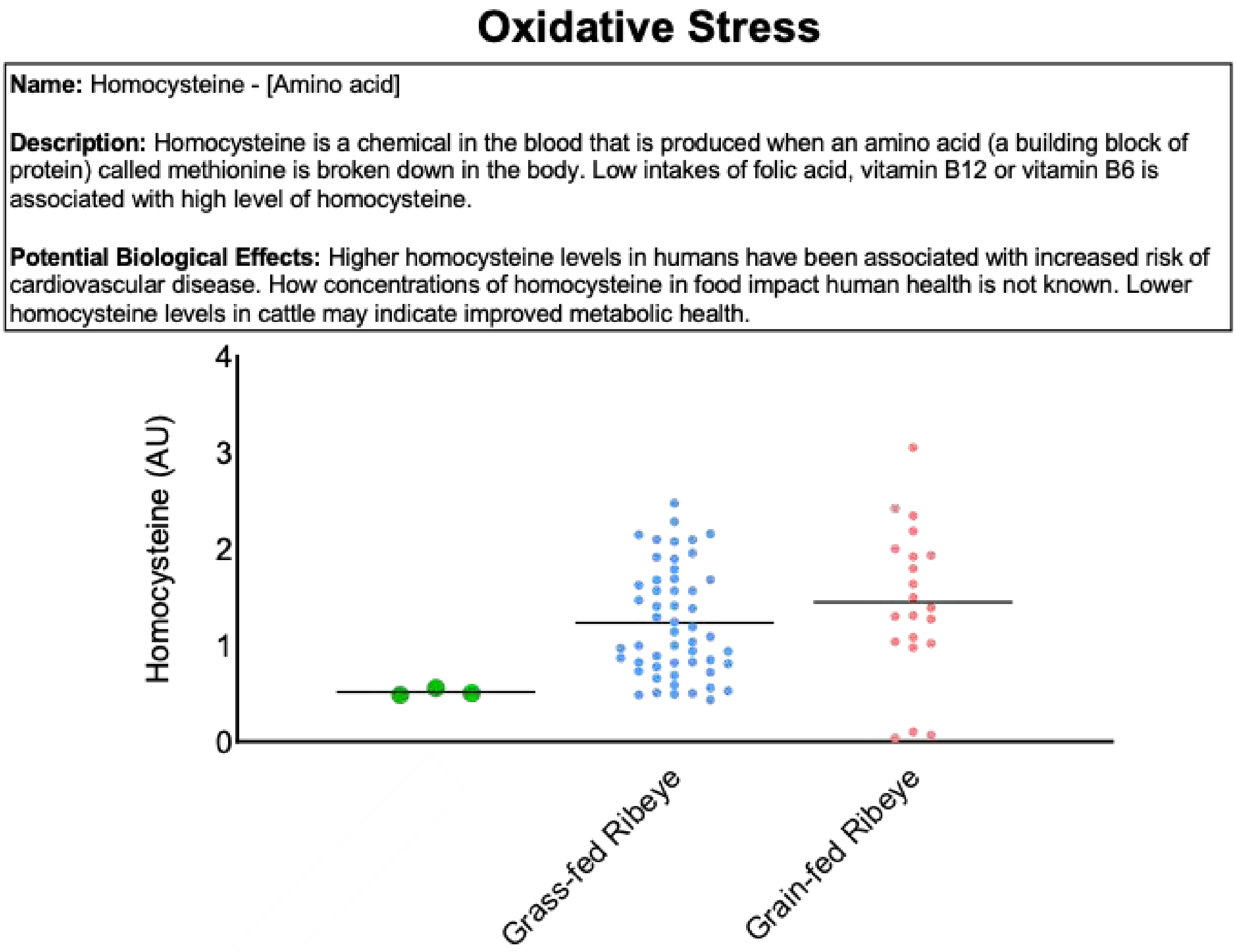


Animal Health Markers

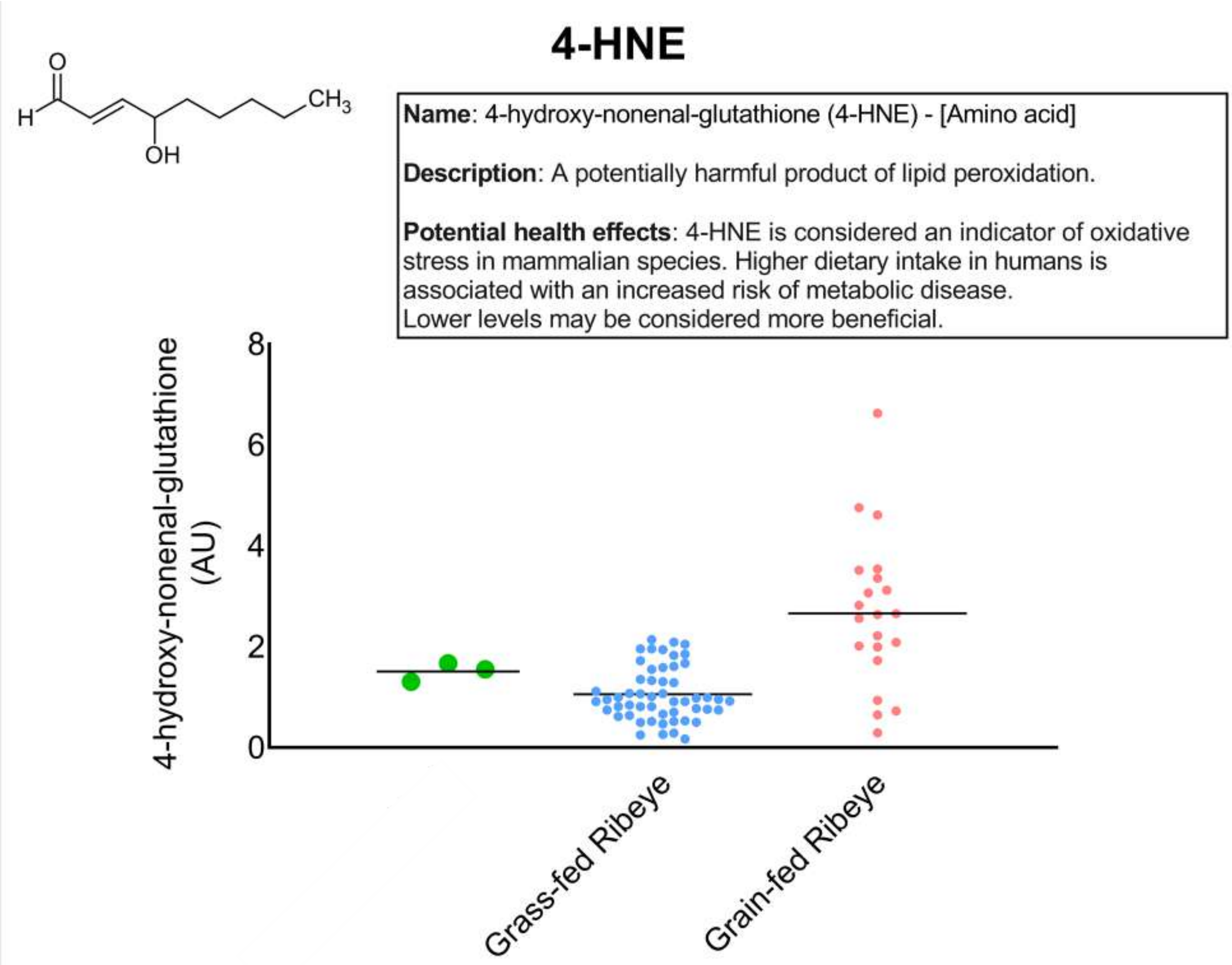
Not unlike human muscle, the muscle (meat) of animals provides many clues regarding the metabolic health of the animal. These include oxidative stress markers, advanced glycation and lipid oxidation end products as well as glutathione. **Advanced lipoxidation end products (ALEs) and advanced glycation end products (AGEs)** are believed to play a pathogenetic role in the development and progression of different oxidative-stress based diseases including diabetes, heart disease, and neurological disorders[47]. AGE/ALEs are proteins or lipids that become glycated after exposure to sugars or are the result of oxidative degradation of lipids. They are known to contribute to increased oxidative stress and inflammation, which are linked to increase risk of diabetes and cardiovascular disease. Lower levels could be considered more favorable. These metabolites are reflective of animal metabolic health but could potentially have implications for consumer health as well[48].



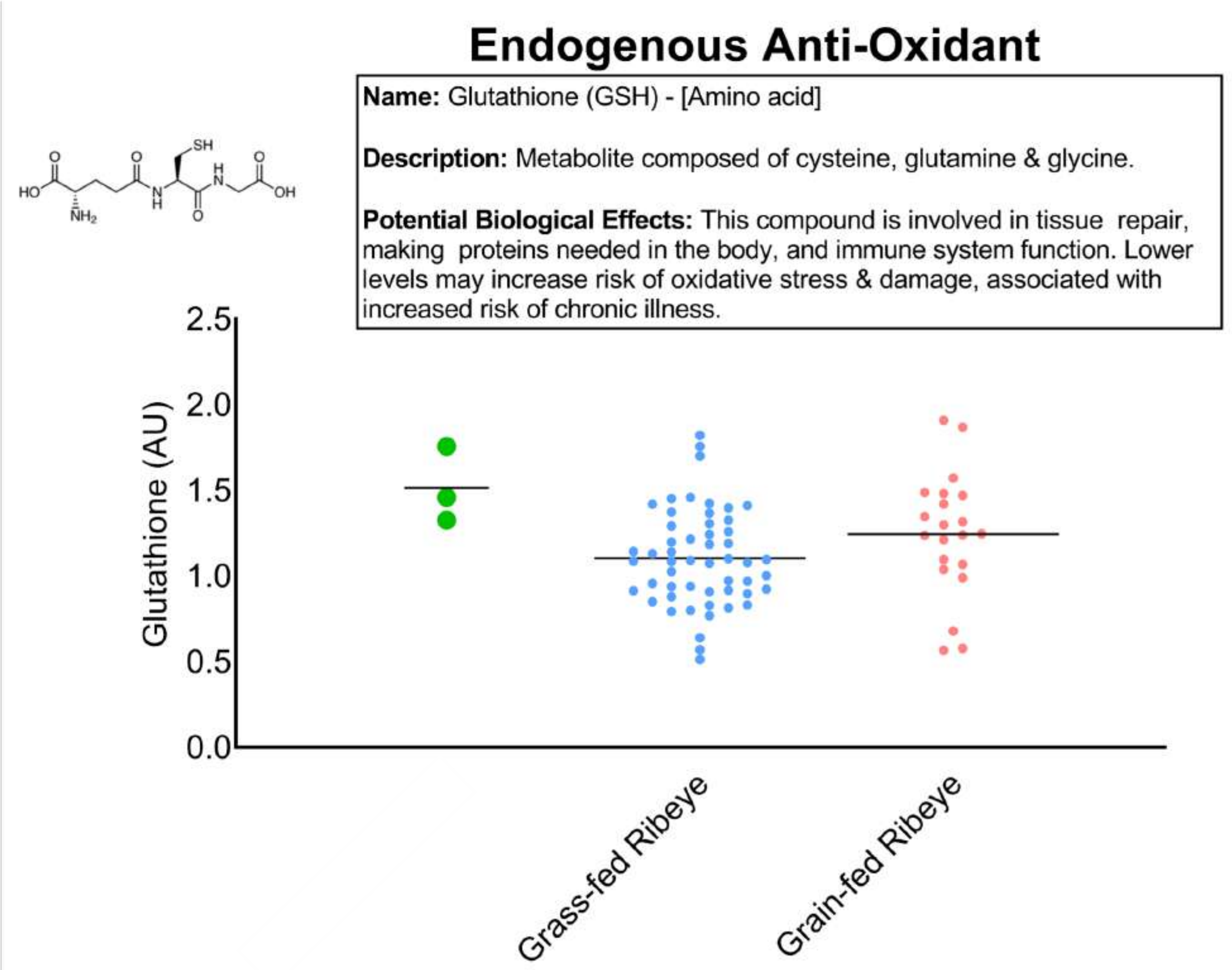
Homocysteine is formed because of methionine metabolism (demethylation of methionine). Ruminants need cobalt for the synthesis of vitamin B12, which is an element found in soil. Low levels of Vitamin B12 can cause an increase in homocysteine levels. Higher levels of homocysteine play a potential role in cardiometabolic diseases and is considered a good biomarker to determine the presence of impairments in cardiometabolic health[49]. Thus, lower levels of homocysteine in ruminants could indicate improved metabolic health of the animal[50]. How concentrations of homocysteine in meat impacts human health is not yet known, though higher homocysteine levels in humans have been associated with increased risk of heart disease[51].



4-hydroxy-nonenal-glutathione (4-HNE), a toxic and most abundant stable end product of lipid peroxidation, has been implicated in the tissue damage, dysfunction, injury associated with aging and other pathological states such as cancer, Alzheimer, diabetes, cardiovascular and inflammatory complications[52]. Further, HNE has been considered as an oxidative stress marker. Lower levels are considered more favorable and could indicate good metabolic health of the animal.

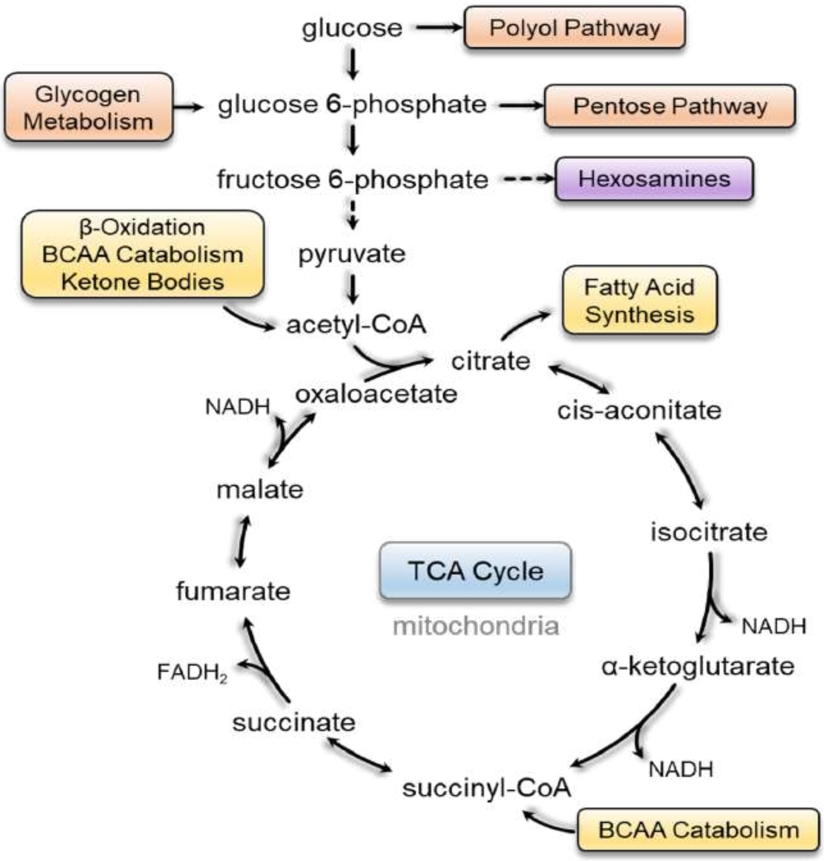


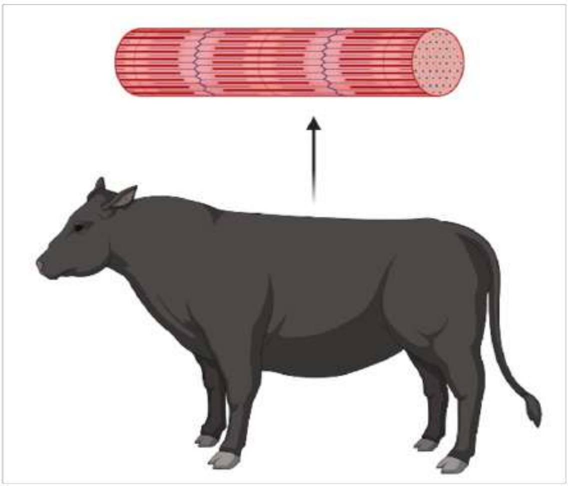
Glutathione (GSH), a metabolite composed of the amino acids, cysteine, glutamine, and glycine, is a major intracellular anti-oxidant in the body of mammals. This compound plays a key role in antioxidant activity and helps with the regeneration of vitamins C and E. It also plays a role in the neutralization of free radicals produced by Phase I liver metabolism of chemical toxins. It is also vital for mitochondrial function and maintenance of mitochondrial DNA[53]. Higher levels may be considered more favorable.



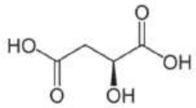
Glucose and Energy Metabolites

The diet of ruminant animals significantly impacts energy metabolism and glucose utilization, and these changes are reflected in their muscle tissue. Grass-fed beef muscle has a higher capacity for oxidative metabolism characterized by increased levels of mitochondrial TCA cycle metabolites[54], while grain-fed animals are typically more reliant on glycolytic metabolism as the feed contains more sugars/carbohydrates. Another factor that can impact reliance on oxidative or glucose metabolism is physical activity. As animals in pasture-based systems are generally more physically active, they display a more aerobic phenotype in the muscle, not unlike humans who are engaged in endurance-based activities[55]. Mitochondria can be considered the metabolic engine of cells and are responsible for generating large amounts of energy under aerobic condition. Higher levels of malate and succinate typically indicate the animal was physically active and in good heart/mitochondrial health.





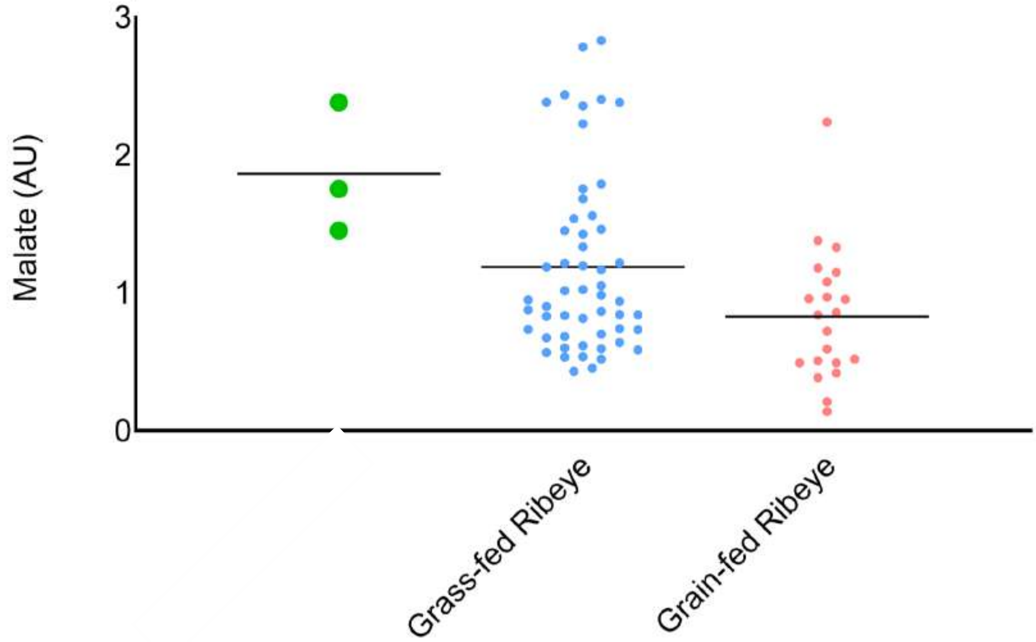
Energy



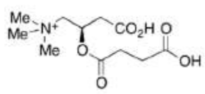
Name: Malate - [Energy]

Description: One of the final intermediates in the TCA cycle, responsible for producing energy through oxidation of acetyl-CoA from fats, carbs, and amino acids.

Potential Biological Effects: Higher levels indicate greater reliance on oxidative metabolism in the muscle, which is common in grass-fed animals, and may confer metabolic health benefits for the animal. Grain-fed animals usually have a greater reliance of glycolysis (breakdown of glucose for energy).



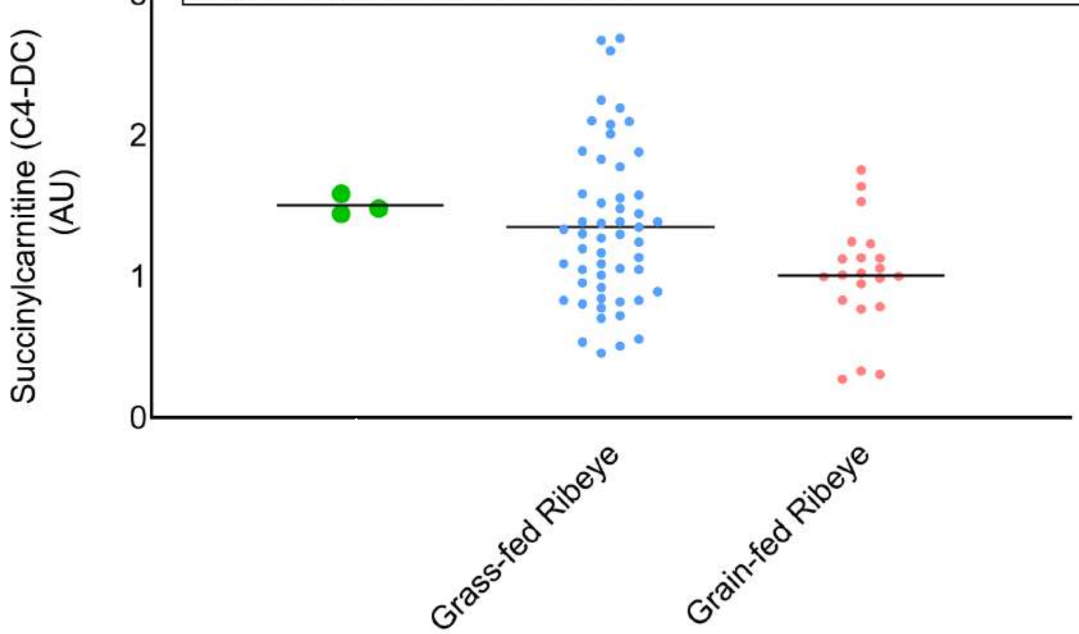
Energy



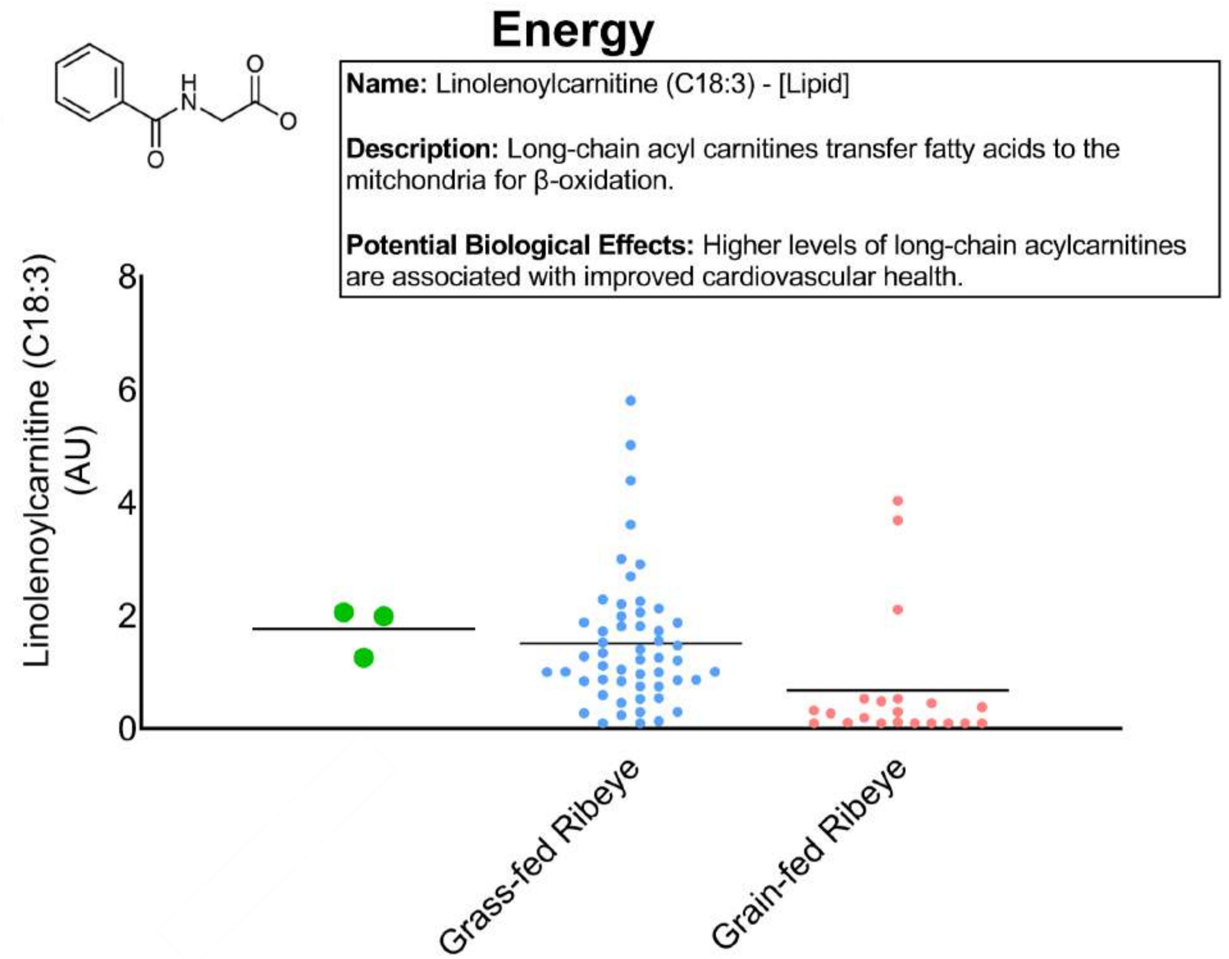
Name: Succinylcarnitine - [Energy]

Description: One of the later intermediates in the TCA cycle, which is responsible for producing energy through oxidation of acetyl-CoA from fats, carbohydrates, and amino acids.

Potential Biological Effects: Higher levels indicate greater reliance on oxidative metabolism in the muscle, which is common in grass-fed animals, and may confer metabolic health benefits for the animal.



Cardiovascular risk can also be related to the fatty acyl glycerol and fatty acyl carnitine content of the meat. In humans, higher levels of long-chain acyl carnitines[56] are associated with improved cardiovascular health. The higher content these long-chain acyl carnitines is likely related to the physical activity of the grass-fed animals, which is in line with the energy (TCA) metabolites, and/or the grazing on fresh forages, which are high in the polyunsaturated omega-3 fatty acid alpha-linolenic acid (ALA).



References

1 Huang, J., Weinstein Stephanie, J., Yu, K., Männistö, S. & Albanes, D. Relationship Between Serum Alpha-Tocopherol and Overall and Cause-Specific Mortality. Circ Res 125, 29-40. doi: 10.1161/CIRCRESAHA.1119.314944 (2019).

2 Provenza, F. D., Meuret, M. & Gregorini, P. Our landscapes, our livestock, ourselves: Restoring broken linkages among plants, herbivores, and humans with diets that nourish and satiate. Appetite 95, 500-519 (2015). https://doi.org:10.1016/j.appet.2015.08.004

3 Dillard, C. J. & German, J. B. Phytochemicals: nutraceuticals and human health. Journal of the Science of Food and Agriculture 80, 1744-1756 (2000). https://doi.org:https://doi.org/10.1002/1097-0010(20000915)80:12<1744::AID-JSFA725>3.0.CO;2-W

4 Choudhari, A. S., Mandave, P. C., Deshpande, M., Ranjekar, P. & Prakash, O. Phytochemicals in cancer treatment: From preclinical studies to clinical practice. Frontiers in pharmacology 10, 1614 (2020).

5 Ismaeel, A. et al. Phytochemicals as Therapeutic Interventions in Peripheral Artery Disease. Nutrients 13, 2143 (2021).

6 Cao, H. et al. Dietary polyphenols and type 2 diabetes: Human Study and Clinical Trial. Critical Reviews in Food Science and Nutrition 59, 3371-3379 (2019). https://doi.org:10.1080/10408398.2018.1492900

7 Fraga, C. G., Croft, K. D., Kennedy, D. O. & Tomás-Barberán, F. A. The effects of polyphenols and other bioactives on human health. Food Funct 10, 514-528 (2019). https://doi.org:10.1039/c8fo01997e

8 Zhang, L., Virgous, C. & Si, H. Synergistic anti-inflammatory effects and mechanisms of combined phytochemicals. The Journal of Nutritional Biochemistry 69, 19-30 (2019). https://doi.org:https://doi.org/10.1016/j.jnutbio.2019.03.009

9 Yin, R. et al. Gut Microbiota, Dietary Phytochemicals, and Benefits to Human Health. Current Pharmacology Reports 5, 332-344 (2019). https://doi.org:10.1007/s40495-019-00196-3

10 Uddin, M. S. et al. Exploring the multimodal role of phytochemicals in the modulation of cellular signaling pathways to combat age-related neurodegeneration. Science of The Total Environment 725, 138313 (2020). https://doi.org:https://doi.org/10.1016/j.scitotenv.2020.138313
Efficacy. Int J Mol Sci 21, 5712 (2020). https://doi.org:10.3390/ijms21165712

11 van Vliet, S., Provenza, F. D. & Kronberg, S. L. Health-Promoting Phytonutrients Are Higher in Grass-Fed Meat and Milk. Frontiers in Sustainable Food Systems 4 (2021). https://doi.org:10.3389/fsufs.2020.555426

12 Lees, H. J., Swann, J. R., Wilson, I. D., Nicholson, J. K. & Holmes, E. Hippurate: the natural history of a mammalian-microbial cometabolite. J Proteome Res 12, 1527-1546 (2013). https://doi.org:10.1021/pr300900b

13 Pallister, T. et al. Hippurate as a metabolomic marker of gut microbiome diversity: Modulation by diet and relationship to metabolic syndrome. Scientific Reports 7, 13670 (2017). https://doi.org:10.1038/s41598-017-13722-4

14 Peperidou, A., Pontiki, E., Hadjipavlou-Litina, D., Voulgari, E. & Avgoustakis, K. Multifunctional Cinnamic Acid Derivatives. Molecules 22, 1247 (2017). https://doi.org:10.3390/molecules22081247

15 Prorok, T., Jana, M., Patel, D. & Pahan, K. Cinnamic Acid Protects the Nigrostriatum in a Mouse Model of Parkinson's Disease via Peroxisome Proliferator-Activated Receptorα. Neurochem Res 44, 751-762 (2019). https://doi.org:10.1007/s11064-018-02705-0

16 Ruwizhi, N. & Aderibigbe, B. A. Cinnamic Acid Derivatives and Their Biological Efficacy. Int J Mol Sci 21, 5712 (2020). https://doi.org:10.3390/ijms21165712

17 Derosa, G., Maffioli, P. & Sahebkar, A. Piperine and Its Role in Chronic Diseases. Adv Exp Med Biol 928, 173-184 (2016). https://doi.org:10.1007/978-3-319-41334-1_8

18 Butawan, M., Benjamin, R. L. & Bloomer, R. J. Methylsulfonylmethane: Applications and Safety of a Novel Dietary Supplement. Nutrients 9 (2017). https://doi.org:10.3390/nu9030290

19 Cortinovis, C. & Caloni, F. Alkaloid-Containing Plants Poisonous to Cattle and Horses in Europe. Toxins (Basel) 7, 5301-5307 (2015). https://doi.org:10.3390/toxins7124884

20 Carrillo, J. A. et al. Integrated metabolomic and transcriptome analyses reveal finishing forage affects metabolic pathways related to beef quality and animal welfare. Scientific reports 6, 25948-25948 (2016). https://doi.org:10.1038/srep25948

21 Li, L. et al. Protective Effect of Stachydrine Against Cerebral Ischemia-Reperfusion Injury by Reducing Inflammation and Apoptosis Through P65 and JAK2/STAT3 Signaling Pathway. Frontiers in Pharmacology 11 (2020). https://doi.org:10.3389/fphar.2020.00064

22 Yu, N., Hu, S. & Hao, Z. Benificial effect of stachydrine on the traumatic brain injury induced neurodegeneration by attenuating the expressions of Akt/mTOR/PI3K and TLR4/NFκ-B pathway. Translational Neuroscience 9, 175-182 (2018). https://doi.org:doi:10.1515/tnsci-2018-0026

23 Cao, T. T. et al. Stachydrine Protects Against Pressure Overload-Induced Cardiac Hypertrophy by Suppressing Autophagy. Cellular Physiology and Biochemistry 42, 103-114 (2017). https://doi.org:10.1159/000477119

24 Wang, M., Shu, Z.-J., Wang, Y. & Peng, W. Stachydrine hydrochloride inhibits proliferation and induces apoptosis of breast cancer cells via inhibition of Akt and ERK pathways. Am J Transl Res 9, 1834-1844 (2017).

25 Swanson, D., Block, R. & Mousa, S. A. Omega-3 fatty acids EPA and DHA: health benefits throughout life. Adv Nutr 3, 1-7 (2012). https://doi.org:10.3945/an.111.000893

26 McAfee, A. J. et al. Red meat from animals offered a grass diet increases plasma and platelet n-3 PUFA in healthy consumers. Br J Nutr 105, 80-89. doi: 10.1017/s0007114510003090 (2011).

27 Sinclair, A. J., Johnson, L., O'Dea, K. & Holman, R. T. Diets rich in lean beef increase arachidonic acid and long-chain omega 3 polyunsaturated fatty acid levels in plasma phospholipids. Lipids 29, 337-343 (1994). https://doi.org:10.1007/bf02537187

28 Sinclair, A. J., O'Dea, K., Dunstan, G., Ireland, P. D. & Niall, M. Effects on plasma lipids and fatty acid composition of very low fat diets enriched with fish or kangaroo meat. Lipids 22, 523-529 (1987). https://doi.org:10.1007/bf02540369

29 Bò, C. D. et al. Horse meat consumption affects iron status, lipid profile and fatty acid composition of red blood cells in healthy volunteers. International Journal of Food Sciences and Nutrition 64, 147-154 (2013). https://doi.org:10.3109/09637486.2012.728198

30 Delgado, G. E. et al. Individual omega-9 monounsaturated fatty acids and mortality-The Ludwigshafen Risk and Cardiovascular Health Study. J Clin Lipidol 11, 126-135.e125 (2017). https://doi.org:10.1016/j.jacl.2016.10.015

31 Chei, C. L. et al. Serum Fatty Acid and Risk of Coronary Artery Disease- Circulatory Risk in Communities Study (CIRCS). Circ J 82, 3013-3020 (2018). https://doi.org:10.1253/circj.CJ-18-0240

32 Micha, R. & Mozaffarian, D. Saturated Fat and Cardiometabolic Risk Factors, Coronary Heart Disease, Stroke, and Diabetes: a Fresh Look at the Evidence. Lipids 45, 893-905 (2010). https://doi.org:doi:10.1007/s11745-010-3393-4

33 Lemaitre, R. N. & King, I. B. Very long-chain saturated fatty acids and diabetes and cardiovascular disease. Curr Opin Lipidol 33, 76-82 (2022). https://doi.org:10.1097/mol.0000000000000806

34 Sellem, L. et al. Impact of Replacement of Individual Dietary SFAs on Circulating Lipids and Other Biomarkers of Cardiometabolic Health: A Systematic Review and Meta-Analysis of Randomized Controlled Trials in Humans. Advances in Nutrition, nmab143 (2021). https://doi.org:10.1093/advances/nmab143

35 Ip, C. et al. Conjugated linoleic acid-enriched butter fat alters mammary gland morphogenesis and reduces cancer risk in rats. J Nutr 129, 2135-2142. doi: 2110.1093/jn/2129.2112.2135 (1999).

36 Aro, A. et al. Inverse association between dietary and serum conjugated linoleic acid and risk of breast cancer in postmenopausal women. Nutr Cancer 38, 151-157. doi: 110.1207/s15327914nc15327382_15327912 (2000).

37 Dewhurst, R. J., Scollan, N. D., Youell, S. J., Tweed, J. K. S. & Humphreys, M. O. Influence of species, cutting date and cutting interval on the fatty acid composition of grasses. Grass and Forage Science 56, 68-74 (2001). https://doi.org:https://doi.org/10.1046/j.1365-2494.2001.00247.x

38 Helzlsouer, K. J. et al. Association Between α-Tocopherol, γ-Tocopherol, Selenium, and Subsequent Prostate Cancer. J Natl Cancer Inst 92, 2018-2023. doi: 2010.1093/jnci/2092.2024.2018 (2000).

39 Das Gupta, S. & Suh, N. Tocopherols in cancer: An update. Mol Nutr Food Res 60, 1354-1363. doi: 1310.1002/mnfr.201500847 (2016).

40 Mangialasche, F. et al. Tocopherols and tocotrienols plasma levels are associated with cognitive impairment. Neurobiol Aging 33, 2282-2290. doi: 2210.1016/j.neurobiolaging.2011.2211.2019 (2012).

41 Delcourt, C. et al. Age-related Macular Degeneration and Antioxidant Status in the POLA Study. Arch Ophthalmol 117, 1384-1390. doi: 1310.1001/archopht.1117.1310.1384 (1999).

42 Ahsan, H., Ahad, A., Iqbal, J. & Siddiqui, W. A. Pharmacological potential of tocotrienols: a review. Nutrition & Metabolism 11, 52 (2014). https://doi.org:10.1186/1743-7075-11-52

43 Chen, J., Yang, Z. & Dong, G. Niacin nutrition and rumen-protected niacin supplementation in dairy cows: an updated review. British Journal of Nutrition 122, 1103-1112 (2019). https://doi.org:10.1017/S0007114519002216

44 Ragaller, V., Lebzien, P., Südekum, K. H., Hüther, L. & Flachowsky, G. Pantothenic acid in ruminant nutrition: a review. Journal of animal physiology and animal nutrition 95, 6-16 (2011).

45 Seck, M. et al. Apparent ruminal synthesis of B vitamins in lactating dairy cows fed diets with different forage-to-concentrate ratios. J Dairy Sci 100, 1914-1922 (2017). https://doi.org:10.3168/jds.2016-12111

46 Duckett, S. K., Neel, J. P. S., Fontenot, J. P. & Clapham, W. M. Effects of winter stocker growth rate and finishing system on: III. Tissue proximate, fatty acid, vitamin, and cholesterol content1. Journal of Animal Science 87, 2961-2970. doi: 2910.2527/jas.2009-1850 (2009).

47 Singh, V. P., Bali, A., Singh, N. & Jaggi, A. S. Advanced glycation end products and diabetic complications. Korean J Physiol Pharmacol 18, 1-14 (2014). https://doi.org:10.4196/kjpp.2014.18.1.1

48 Uribarri, J. et al. Advanced glycation end products in foods and a practical guide to their reduction in the diet. J Am Diet Assoc 110, 911-916.e912 (2010). https://doi.org:10.1016/j.jada.2010.03.018

49 Prasad, K. Homocysteine, a Risk Factor for Cardiovascular Disease. Int J Angiol 8, 76-86 (1999). https://doi.org:10.1007/bf01616850

50 Kiliçkap, A. & Kozat, S. Research of serum homocysteine levels in healthy cows. J Vet Sci Anim Husb 5, 103 (2017).

51 Bostom, A. G. et al. Nonfasting plasma total homocysteine levels and all-cause and cardiovascular disease mortality in elderly Framingham men and women. Archives of Internal Medicine 159, 1077-1080 (1999).

52 Shoeb, M., Ansari, N. H., Srivastava, S. K. & Ramana, K. V. 4-Hydroxynonenal in the pathogenesis and progression of human diseases. Curr Med Chem 21, 230-237 (2014). https://doi.org:10.2174/09298673113209990181

53 Minich, D. M. & Brown, B. I. A review of dietary (phyto) nutrients for glutathione support. Nutrients 11, 2073 (2019).

54 Apaoblaza, A. et al. Muscle from grass- and grain-fed cattle differs energetically. Meat Sci 161, 107996 (2020). https://doi.org:10.1016/j.meatsci.2019.107996

55 Kelly, R. S., Kelly, M. P. & Kelly, P. Metabolomics, physical activity, exercise and health: A review of the current evidence. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1866, 165936 (2020). https://doi.org:https://doi.org/10.1016/j.bbadis.2020.165936

56 Bhupathiraju, S. N. et al. Dietary Patterns among Asian Indians Living in the United States Have Distinct Metabolomic Profiles That Are Associated with Cardiometabolic Risk. J Nutr 148, 1150-1159 (2018). https://doi.org:10.1093/jn/nxy074