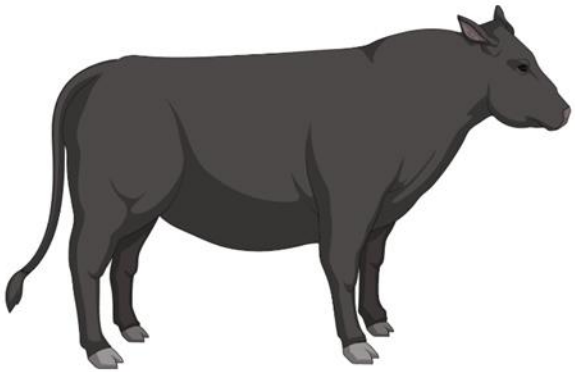
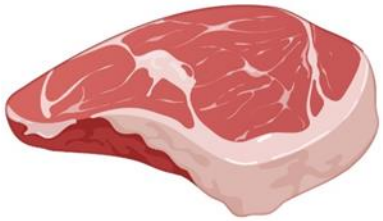
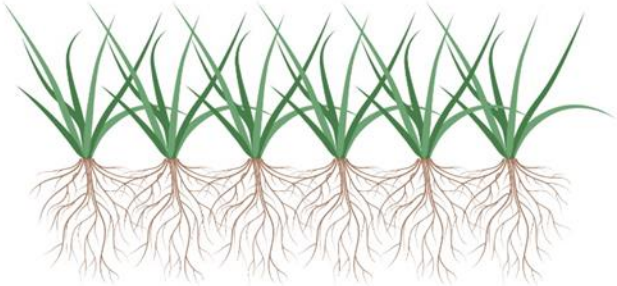
Project Report

Grass-Fed vs. Grain-Fed Beef Metabolomics







Utah State University

Dixon Water Foundation

USDA ARS

Duke University

Study Overview

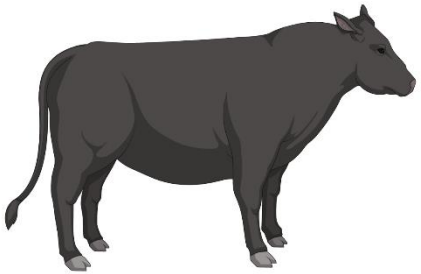
Study Objective

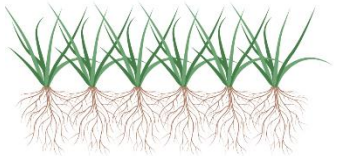
The goal of this study was to determine the differences in metabolomes (nutrient profiles and animal health markers) of grass-fed vs grain fed beef.

Study Design

Grain-finished beef was obtained from a Midwest feedlot (South Dakota, USA). **Grass-finished beef** was obtained from Southern Idaho, USA were cattle are herded on mountain pastures during the grazing season.

Group ID	Treatment	Description	N
Bf_Grass	Grass	Grass fed bovine meat; ground and cooked	18
Bf_Grain	Grain	Grain fed bovine meat; ground and cooked (Control)	18

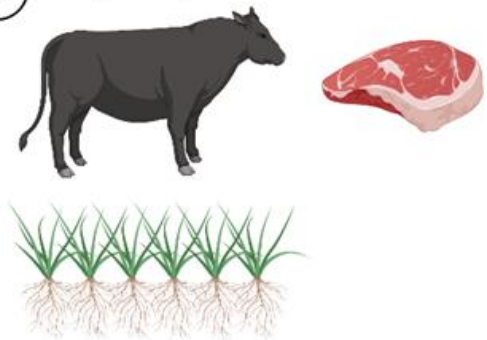




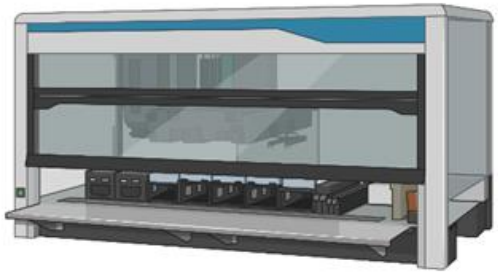
or

Study Workflow

a Sample acquisition



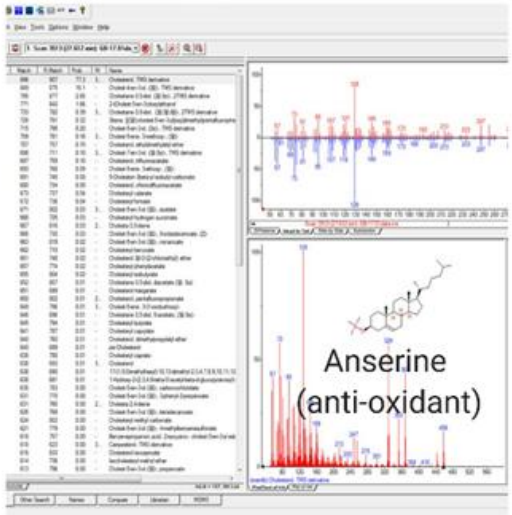
b Sample processing



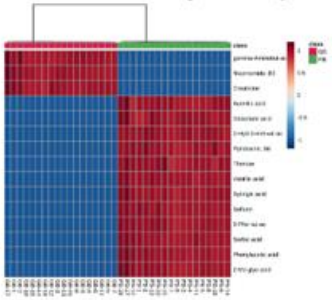
c Mass-spec analysis

Liquid Chromatography with tandem mass spectrometry (LC/MS/MS)

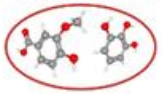
d Metabolite identification



e Bioactivities and pathway analysis



Polyphenols



Amino Acids

Clustering of metabolites into nutrient classes

F Data Intepretation

Amino Acid Metabolism
- Bioactive intermediates, glutathione (anti-oxidant) metabolism, inflammatory mediators, urea metabolism.

Phytochemicals
- Flavonoids, phenolic compounds, anti-oxidants.

Fatty acid metabolism
- Omega 3/6 fatty acids, cholesterol metabolism, bile acid metabolism, bioactive lipids.

Vitamin & Nucleotide Metabolism
- DNA damage, FAD metabolism, ascorbate (Vitamin C) metabolism, folate metabolism, tocopherols (Vitamin E), B12, biotin etc.

Microbiome Metabolism
- Choline/carnitine, short-chain & medium chain fatty acids, secondary bile acids.

FOOD3 PubChem

Statistical Summary

Untargeted Metabolomics

Statistical Comparisons	
Total Biochemicals Identified	578
Total Biochemicals $p<0.05$	377 (65%)
↑Grass	286
↑Grain	91
Total Biochemicals $0.05<p<0.10$	26 (4%)
↑Grass	18
↑Grain	8

Complex Lipid Analysis

Statistical Comparisons	
Total Biochemicals Identified	998
Total Biochemicals $p<0.05$	530 (53%)
↑Grass	307
↑Grain	223
Total Biochemicals $0.05<p<0.10$	86 (9%)
↑Grass	47
↑Grain	36

***Higher does not per se mean better, it depends on the biochemical.**

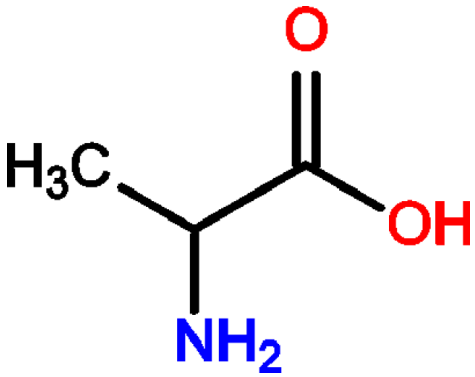
Data Explanation

Pathway	Compound Name	Fold of Change Bi_Grass Bi_Grain
Alanine and Aspartate Metabolism	alanine	1.08
	N-acetylalanine	0.88
	N-methylalanine	0.83
	aspartate	1.11
	N-acetylaspartate (NAA)	0.88
	asparagine	0.97
	N-acetylasparagine	1.26
	hydroxyasparagine**	0.48

Green = ↑Grass Fed

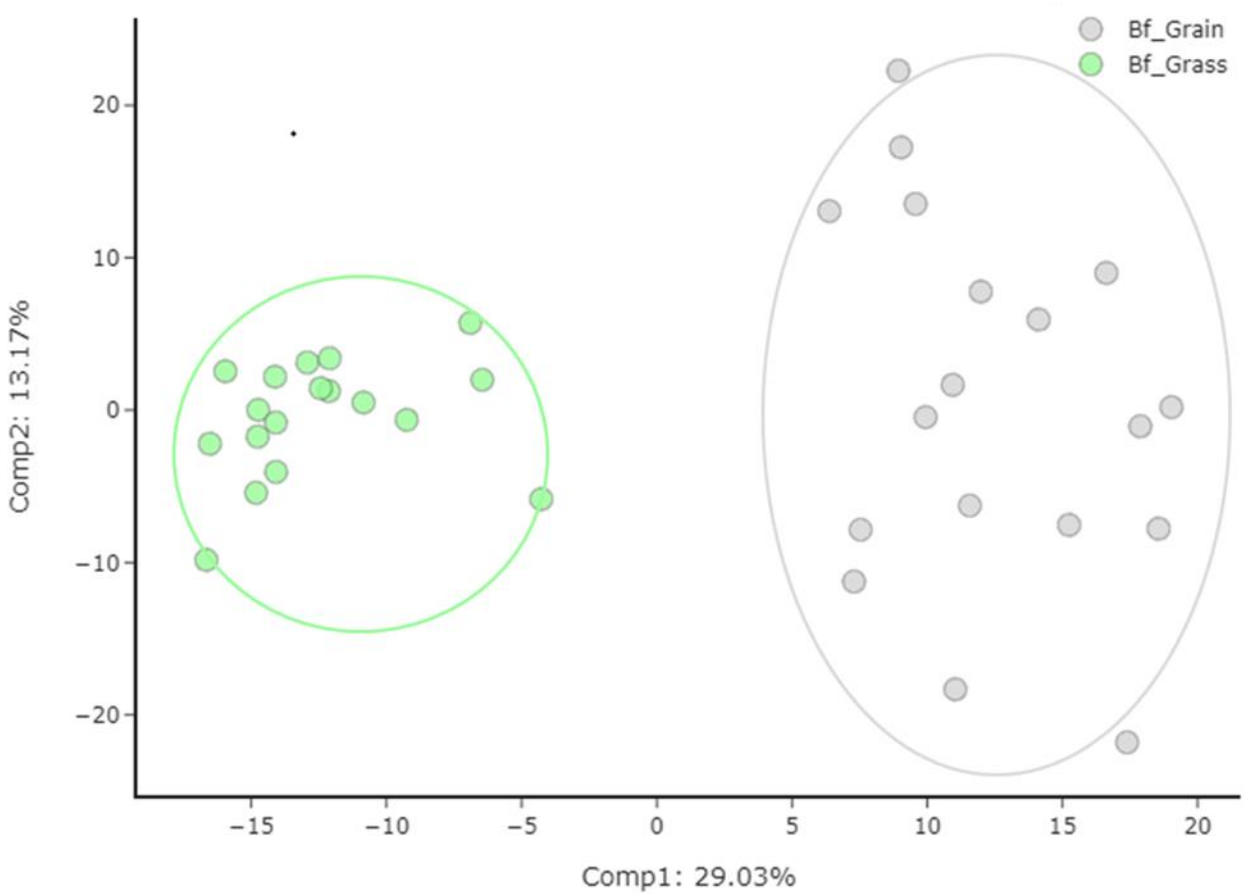
Red = ↑Grain Fed

The number indicates how much higher (e.g., alanine is 1.08 higher in grass-fed bison vs grain-fed bison).



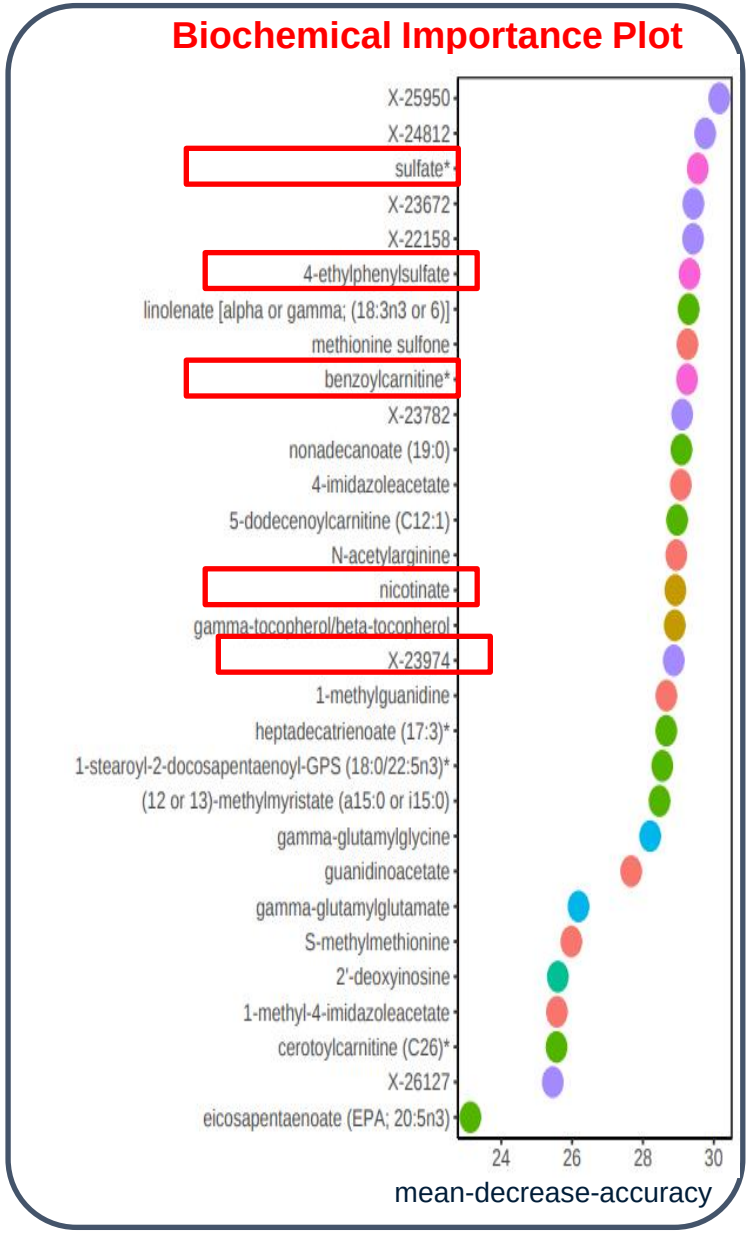
Overview of the Dataset: Principal Component Analysis

Untargeted Metabolomics



Grass-fed beef isn't grain-fed beef and grain-fed beef isn't grass-fed beef….
There is a clear separation between groups.

Overview of the Dataset: Random Forest



Random Forest Confusion Matrix

Actual Group	Predicted Group: Beef Grass Fed	Predicted Group: Beef Grain Fed	Class Error
Beef Grass Fed	18	0	0%
Beef Grain Fed	0	18	0%

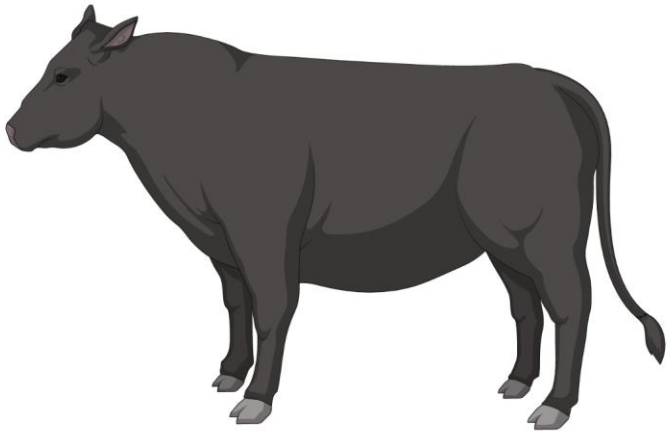
Predictive accuracy = 100%

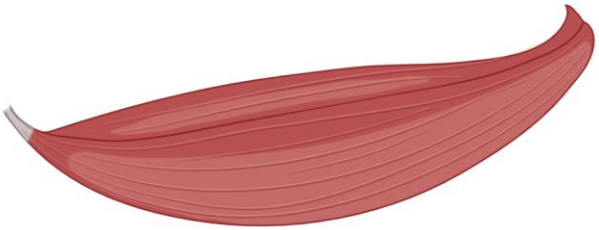
- Amino acid
- Vitamins
- Lipids
- Phytochemicals/ xenobiotics
- Unknown compound

The 30 top ranking biochemicals in the importance plot suggest key differences in

- vitamin metabolism
- phytochemical metabolism
- lipid metabolism

Animal Health Markers



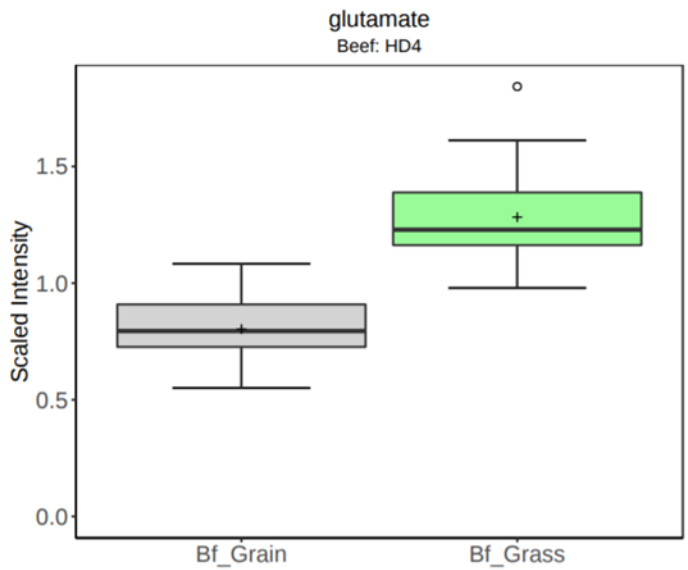


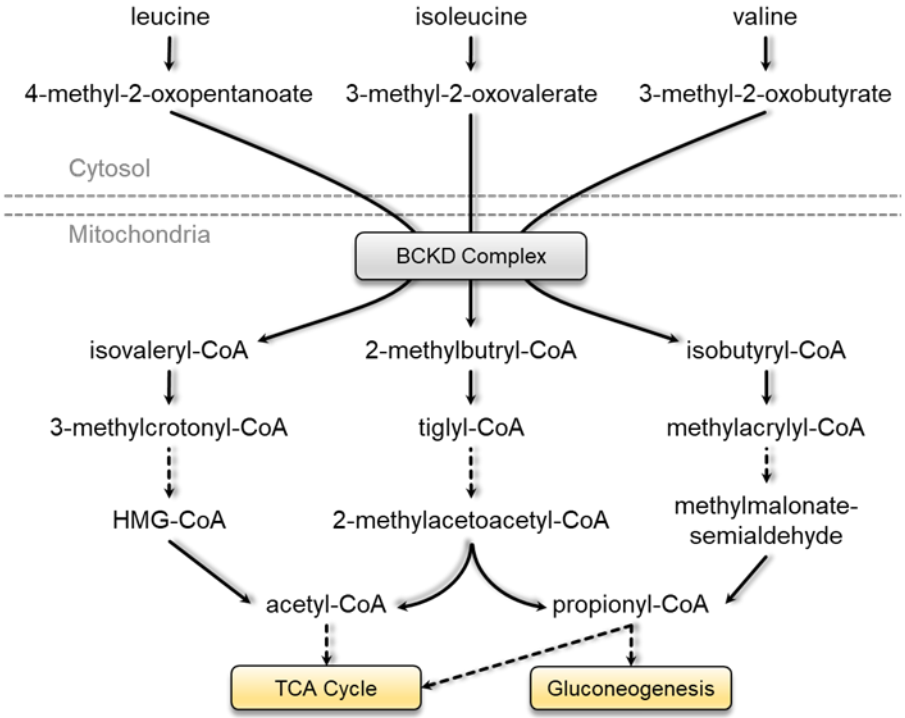
Not unlike human muscle, the muscle of animals provide many clues regarding the metabolic health of the animal.

Energy Metabolism

Sub Pathway	Biochemical Name	Fold difference Bf_Grass / Bf_Grain
Fructose, Mannose and Galactose Metabolism	fructose	1.38
	mannitol/sorbitol	1.45
TCA Cycle	citrate	1.65
	aconitate [cis or trans]	2.42
	succinylcarnitine (C4-DC)	1.23
	succinate	1.34
	fumarate	0.50
	malate	1.38

Mitochondria of pastured beef shows energetic phenotypes not unlike those found in athletic humans (that other mammal)



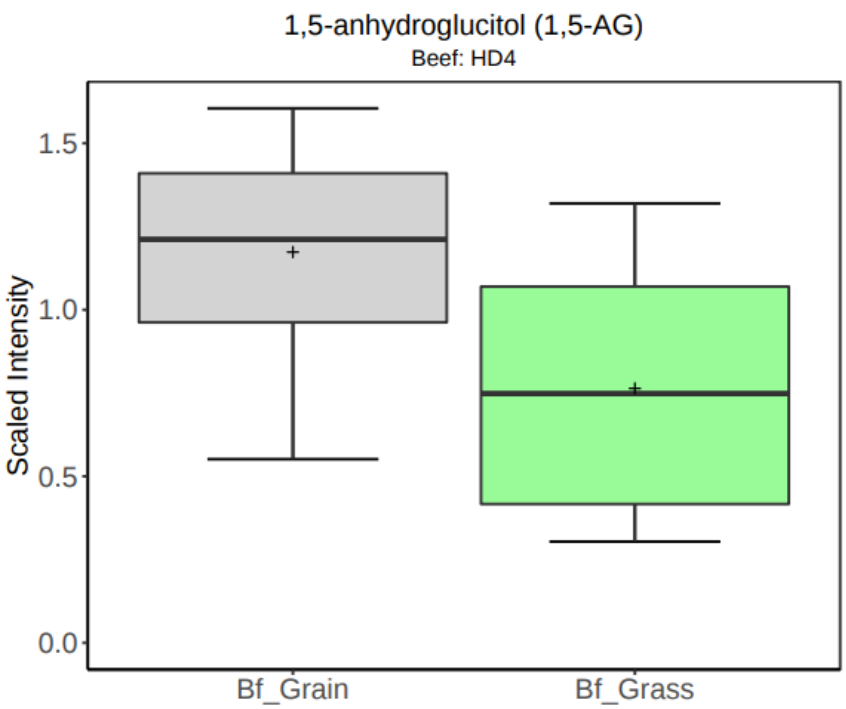


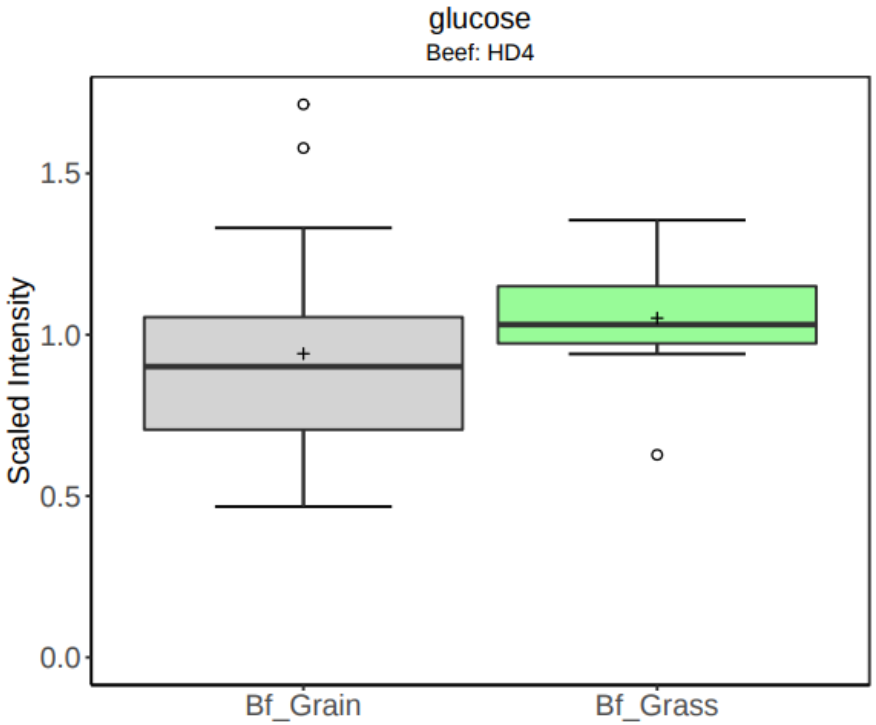
Sub Pathway	Biochemical Name	Fold difference Bf_Grass / Bf_Grain
Urea Cycle	urea	1.28
	ornithine	2.02
	citrulline	2.91
	homoarginine	2.61
Branched Chain Amino Acid Metabolites	isovalerylglycine	1.79
	beta-hydroxyisovaleroylcarnitine	1.87
	2-methylbutyrylcarnitine (C5)	1.66
	tiglylcarnitine (C5:1-DC)	1.51
	isobutyrylcarnitine (C4)	1.26
	3-hydroxyisobutyrate	1.26

Higher carnitine metabolites may be indicative of increased muscle protein content of grass-fed animals.

The higher content of these amino acids is likely the result of increased physical activity of the grass-fed animals, which is in line with the energy (TCA) metabolites.

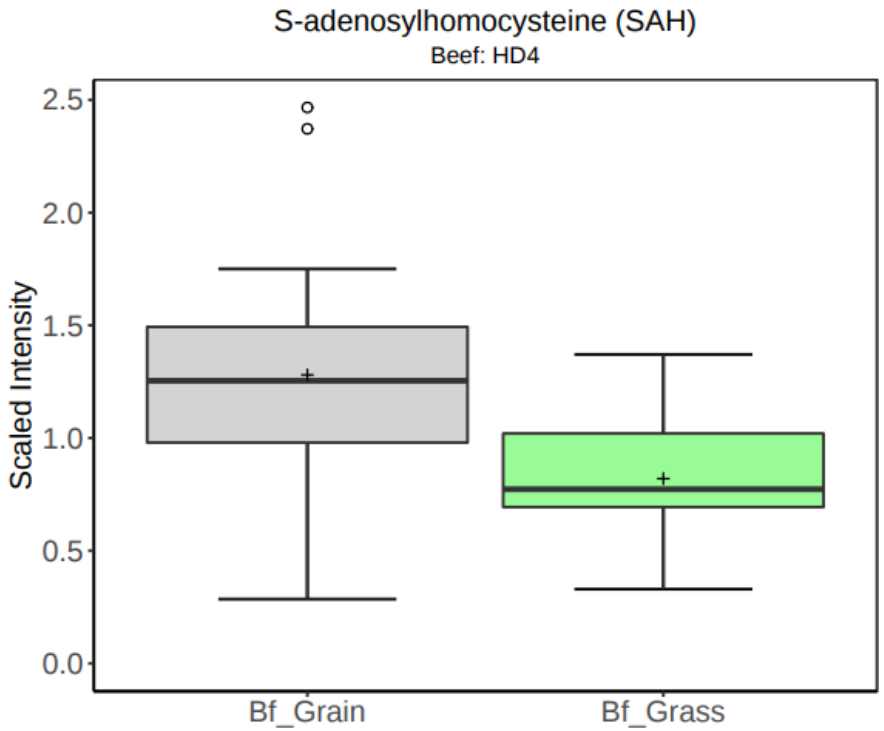
Glucose control

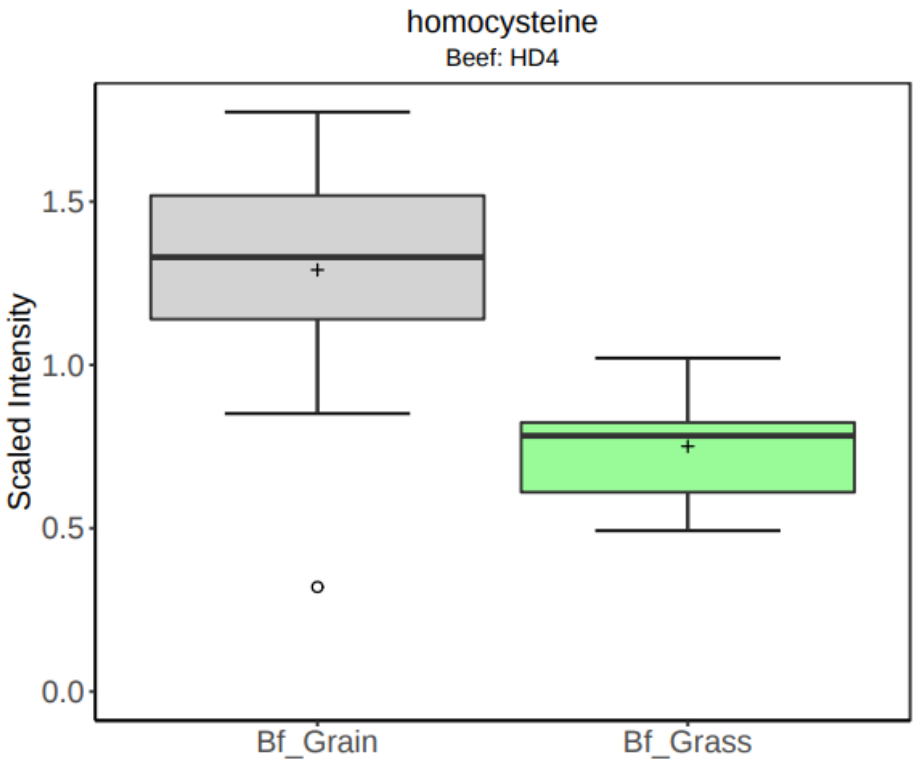




1,5-AG is an indicator of longer-term glucose levels like Hb1AC in humans and higher levels are associated with decreased glucose metabolic health. Lower amounts are therefore considered beneficial for animal health. Glucose levels and its intermediates in glycolysis were higher in grass-fed beef, this could indicate higher sugar/carbohydrate levels in the forage. That is somewhat suprising given that grain-based rations are typically higher in sugars.

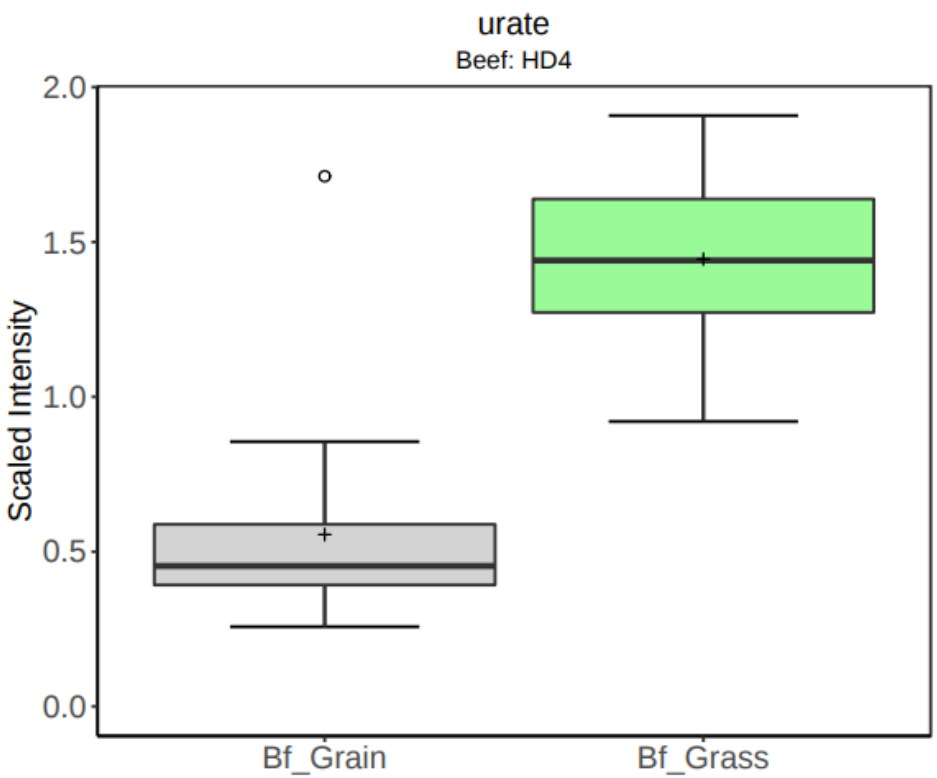
Homocysteine

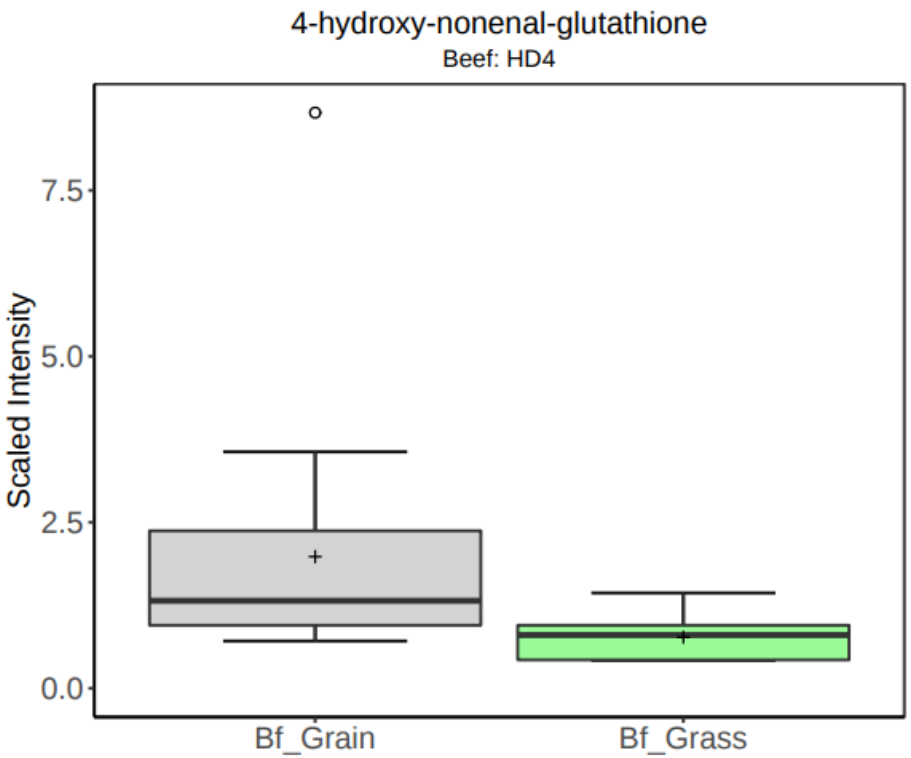




Homocysteine and S-adenosylhomocysteine were higher in grain fed patties, which could have implication for cardiovascular disease (Prasad, 1999). This can be viewed as a further indication that the grass-fed animals were metabolically healthier than the grain-fed animals. Higher homocysteine levels in humans have been associated with increased risk of heart disease in humans (Bostom et al., 1999)

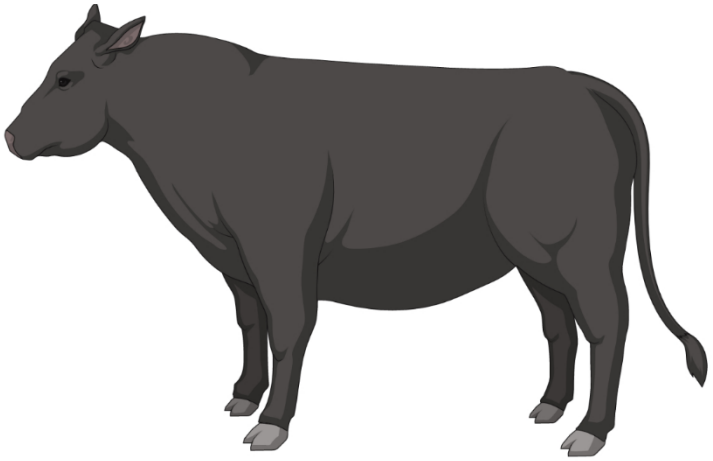
Oxidative Stress Markers

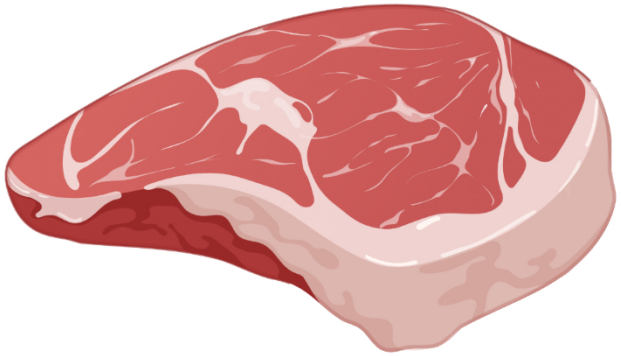




Urate is a major intracellular anti-oxidant and higher levels indicate improved anti-oxidant status in the animal. Higher levels of 4-hydroxy-nonenal-glutathione indicate higher oxidative stress. Taken together these two metabolites indicate less oxidative stress and is in line with other indicators of diminished metabolic health.

Nutrient density

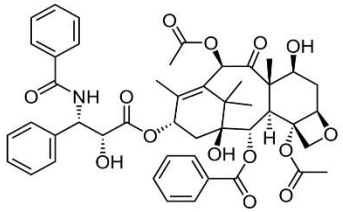




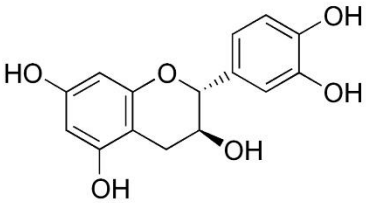
Phytochemicals, vitamins & minerals, omega-3 fats, triaglycerols, amino acids

Phytochemicals

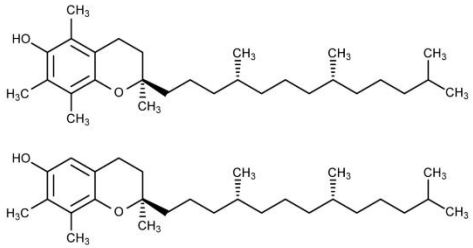
Plants respond biochemically to sunlight, moisture, nutrients, other plants, and herbivores by producing phytochemicals, which are incorporated into meat



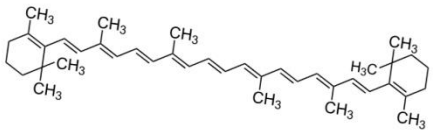
Terpenes



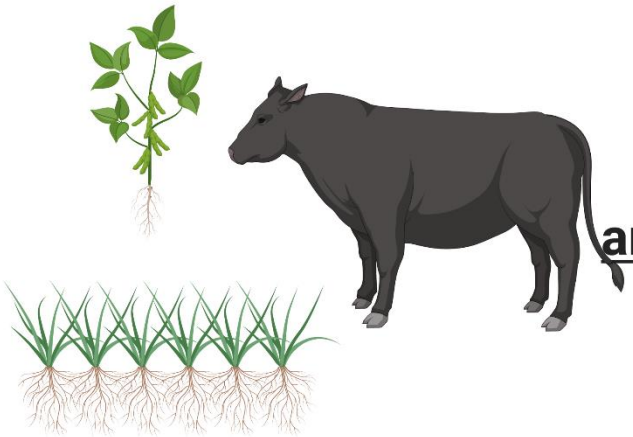
Phenols



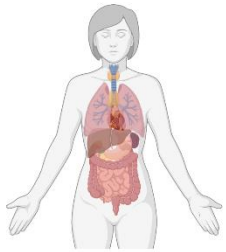
Tocopherols



Carotenoids

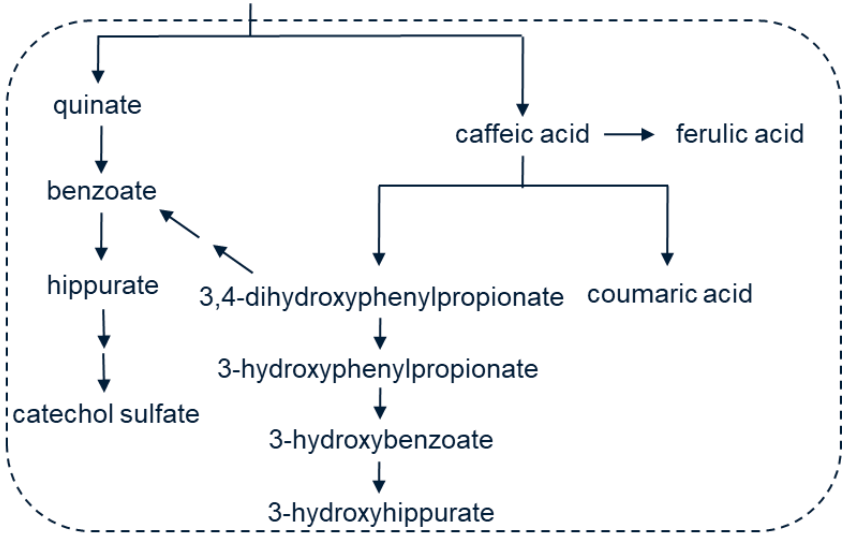


<u>Phytochemicals: potentially anti-inflammatory, anti-bacterial, anti-oxidant etc.</u>

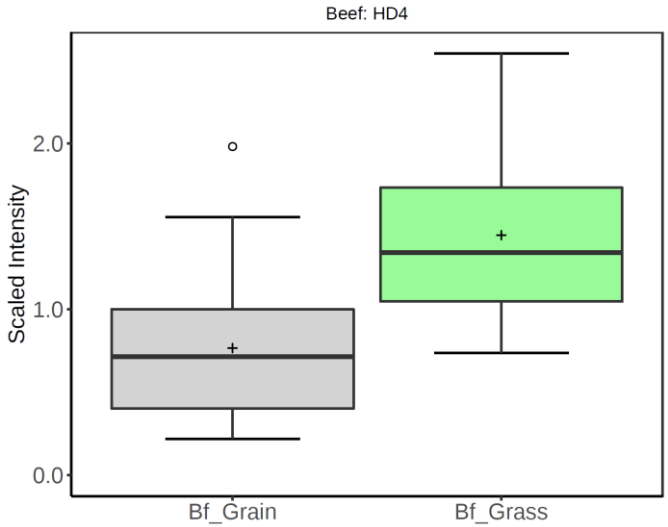


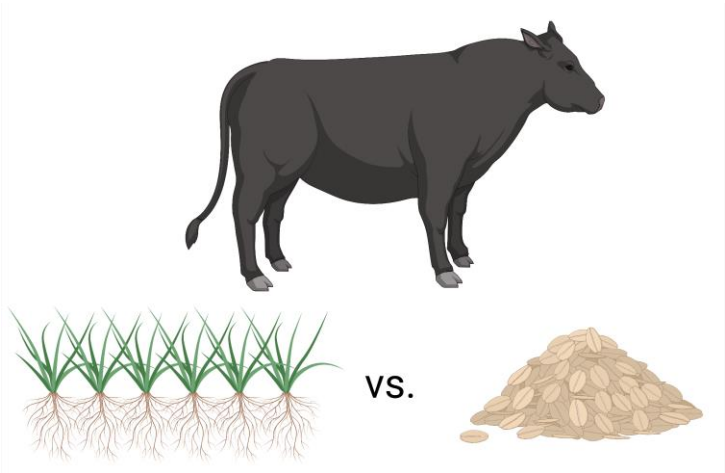
Phytochemicals

Plant phenolic compounds



Hippurate

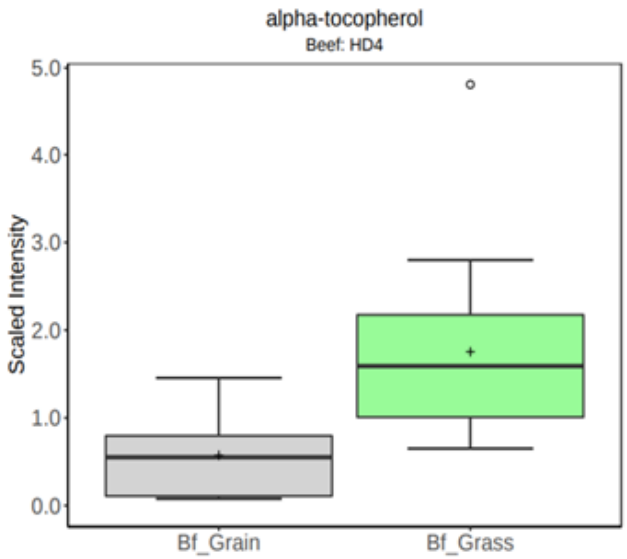




On average 2.5-fold higher in pastured animals

Sub Pathway	Biochemical Name	Fold difference Bi_Grass / Bi_Grain
Phytochemical Metabolism	hippurate	1.89
	catechol sulfate	2.65
	p-cresol sulfate	2.17
	4-ethylphenylsulfate	7.07
	cinnamoylglycine	1.38
	2,8-quinolinediol	0.36
	homostachydrine	4.30
	stachydrine	2.20

Vitamin metabolites



Tocopherols are vitamin E precursors best known for their anti-oxidative effects. Alpha-tocopherol is most abundant in forage and its abundance was 3-fold higher in beef. The higher level of gamma/beta tocopherol in grain-fed beef is the result of the vitamin/mineral additives to total mixed rations.

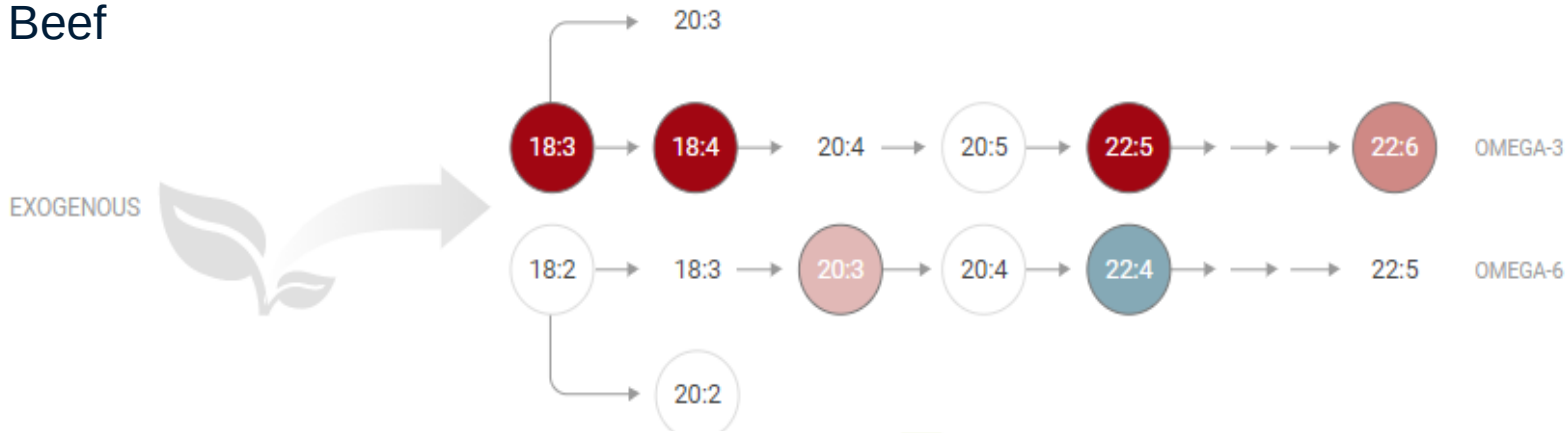
Sub Pathway	Biochemical Name	Fold difference Bf_Grass / Bf_Grain
Vitamin B3 metabolism	niacin	9.41
	nicotinamide	0.63
	nicotinamide ribonucleotide (NMN)	0.85
	nicotinamide adenine dinucleotide (NADH)	1.20
Pantothenate and CoA Metabolism	pantothenate	0.76
Ascorbate and Aldarate Metabolism	dehydroascorbate	1.63
Tocopherol Metabolism	alpha-tocopherol	3.05
	gamma-tocopherol/beta-tocopherol	0.07
Choline Metabolism	choline	1.24
	glycerophosphorylcholine (GPC)	1.23
	glycerophosphoserine*	1.27

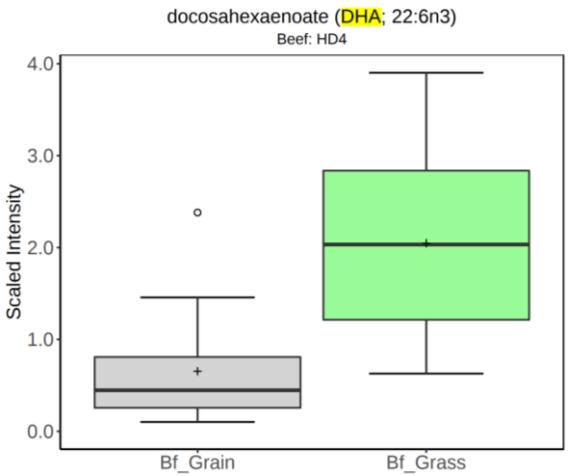
Niacin (the main form of Vitamin B3 in foods) was 9-fold higher in the grass-fed beef. Niacin is known to help promote a healthy nervous system, digestive system, and skin. Nicotinamide (higher in grain-fed beef) is a common supplemental from of B3 and only present in small amounts in nature.

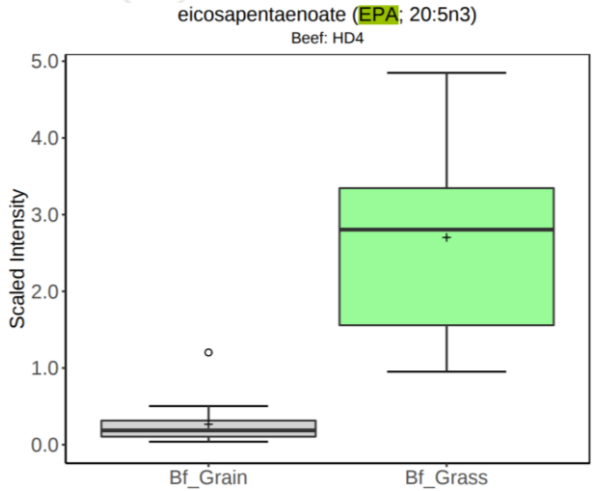
Choline was 1.2 fold higher in grass-fed beef. Choline is an essential nutrient that is important for brain function, muscle function, and liver function (Zeisel & da Costa, 2009).

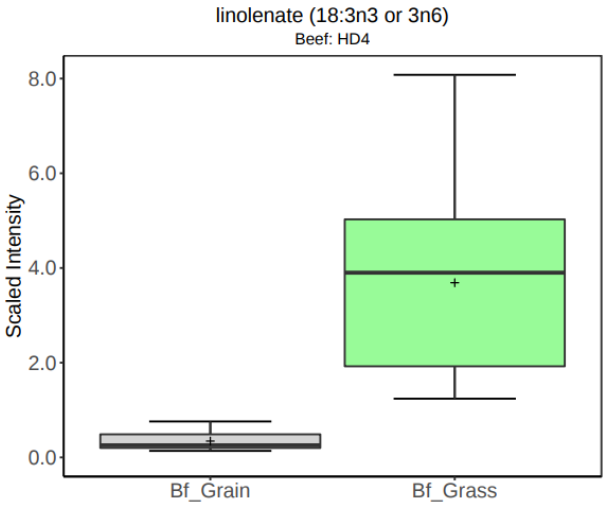
Omega-3s

Beef



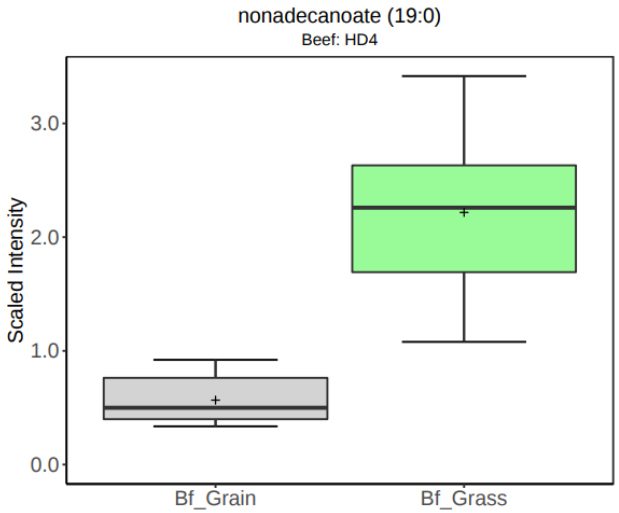


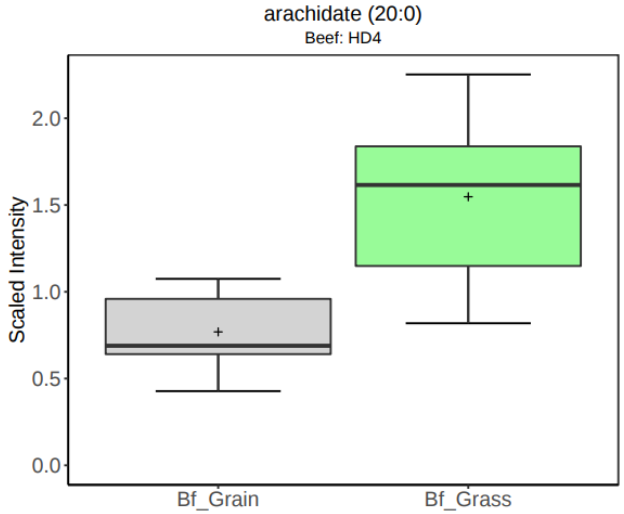


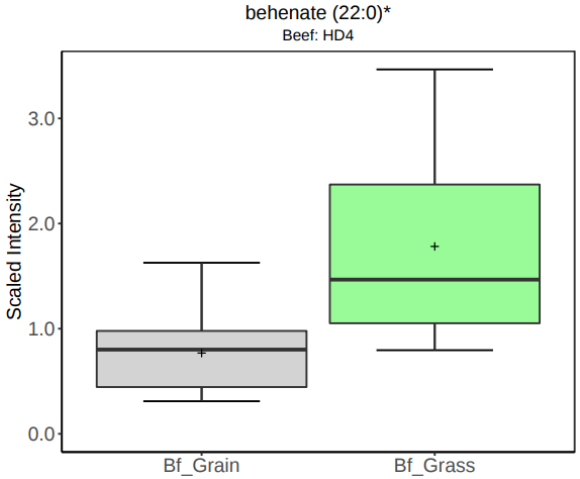


Docosahexanoic acid (DHA), Eicosapentaenoic acid (EPA) and Linolenic acid (ALA) are omega-3 fatty acids. They have well-known health benefits including being anti-inflammatory and anti-oxidants. They may help lower the risk of getting heart disease, cancer, and liver diseases and could help improve brain function (Swanson et al., 2012). DHA, EPA, and ALA were 3-, 10-, and 10-fold higher in grass-fed beef.

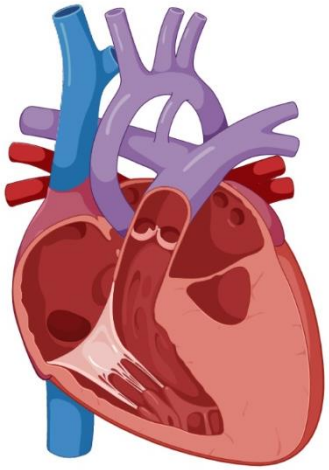
Very long-chain saturated fatty acids



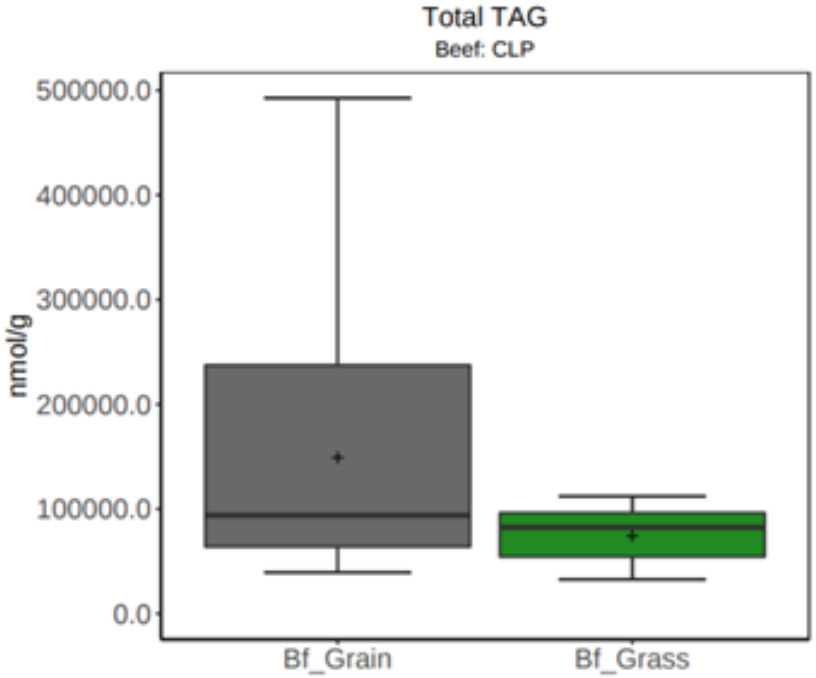




Similar to the long-chain polyunsaturated (omega-3) fatty acids, very long-chain saturated fatty acids also became enriched in grass-fed beef. In contrast to other saturated fats, these long-chain saturated fats are actually associated with a decreased risk of cardiovascular disease. That these fatty acids also become enriched is less recognized by consumers and the scientific community.



Glycerides



Within beef samples, TAG metabolites were significantly reduced in grass- compared to grain-fed samples and long-chain acyl carnitines were elevated.

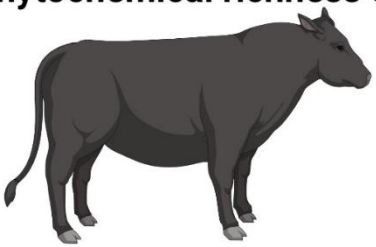
In humans, lower TAGs (Nordestgaard & Varbo, 2014) and higher long-chain acyl carnitines (Bhupathiraju et al., 2018) are associated with improved cardiovascular health.

Based on data in non-human mammals (Nordestgaard, 2016), we consider these findings further reflect that the grass-fed cattle were metabolically healthier.

Sub Pathway	Biochemical Name	Fold of Change Bi_Grass / Bi_Grain
Cholesteryl Esters	Total CE	0.86
Diacylglycerols	Total DAG	**1.24**
Triacylglycerols	Total TAG	**0.50**
Monoacylglycerols	Total MAG	**1.63**
Fatty Acid Metabolism (Acyl Carnitine, Long Chain Saturated)	myristoylcarnitine (C14)	1.10
	pentadecanoylcarnitine (C15)*	0.84
	palmitoylcarnitine (C16)	**1.48**
	margaroylcarnitine (C17)*	1.05
	stearoylcarnitine (C18)	0.76
	Arachidoylcarnitine (C20)*	0.51
	behenoylcarnitine (C22)*	**1.62**
	cerotoylcarnitine (C26)*	**9.07**

Summary

Improved phytochemical richness of feed + enhanced ability to engage in activity



- ↑ Metabolic Health of Animal
  - ↑ Improved mitochondrial/energetic health
  - ↑ Glucose metabolic health
  - ↓ Homocysteine
    improved cardiovascular health
  - ↓ Oxidative stress
    better muscle health/potential meat quality
- ↑ Omega-3 fatty acids
  - improved brain and cardiovascular health
- ↑ Long-chain saturated fatty acids
  - improved cardiovascular health

- ↑ Phytochemical/anti-oxidants
  - ↑ Polyphenols (anti-oxidants)
  - ↑ Carotenoids (vitamin A)
  - ↑ Tocopherols (vitamin E)
    (all have anti-inflammatory effects)
- ↓ Triaglycerols
  - improved cardiovascular health
- ↑ Niacin and choline
  - improved cardiovascular health
- ↑ Carnitine metabolites
  - important for heart and brain function

Healthier, soil = healthier animal = healthier meat?

We observed casual relationships with better soil health (SOM, TEC, and minerals) in the grazing systems.

Does not per se indicate that grain-fed beef is therefore unhealthy, but comparatively, pasture-raised beef certainly looks healthier “on paper”.