Effects of Different Production Systems on the Nutrient Density of Beef

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Key words: beef; metabolomics; grass-fed; grain-fed; nutrient density.

Abstract

As concerns regarding beef production systems on human health and animal welfare become more apparent, consumer interest in pasture-raised livestock has been steadily on the rise in the US. Such interest has increasingly led to questions about potential nutritional composition differences in beef from different production systems, such as grass-fed beef and grain-fed beef. The goal of this work was to conduct untargeted metabolomics analysis on a broad range of samples from the US beef supply chain to provide insight into how different finishing systems impact the beef metabolome and nutrient density. Here, we found that 191 out of 802 profiled compounds were different between grass-fed and grain-fed ribeye steaks (all, $p<0.05$), with phytochemicals, vitamins, lipid, and amino acid metabolites emerging as the most discriminatory metabolite classes. On average, phytochemicals were 1.7-fold higher in grass-fed beef compared to grain-fed beef ($p<0.05$) with considerable variation (4.4-fold) amongst individual farms, particularly within grass-fed beef systems. Alpha-tocopherol was 2-fold elevated in grass-fed beef, while nicotinamide was 1.3-fold elevated in grain-fed beef, respectively ($p<0.05$). We also observed that 4-hydroxy-nonenal-glutathione, a common marker of oxidative stress, was 2.7-fold elevated in grain-fed beef samples ($p<0.05$), with a 20-fold variation across individual farms. Future work will identify the source(s) of variation and best practices in beef systems to improve beef nutrient density and animal metabolic health.

Introduction

Consumer interest in pasture-raised livestock has been steadily on the rise in the US, in part due to concerns about current livestock production systems on human, environmental, and animal health. Although grass-fed beef has been found to have improved omega-3:omega-6 ratios and higher quantities of conjugated linoleic acid (CLA) (Nogoy et al. 2022), few attempts have been conducted to see if further differences exist in the biochemical complexity of beef. Preliminary data indicate that cattle finished on pasture and forages accumulate a wide variety of phytochemicals and biochemicals such as terpenes, phenols, carotenoids, and tocopherols in their meat (van Vliet, Provenza, and Kronberg 2021). Subsequently, these chemicals may benefit animal metabolic health and raise the nutrient quality of beef. However, most prior studies only investigated a limited number of compounds in beef and were predominantly conducted in European countries with limited work performed in North American systems (Prache et al. 2022). Thus, the goal of this study was to use untargeted metabolomics approaches to probe the relative amounts of metabolites in a large number of beef samples from grass-fed cattle and grain-fed cattle in the US supply chain.

Methods

Selection of Producers

A program with the Bionutrient Institute (https://www.bionutrientinstitute.org/) was implemented to provide shipping kits to farmers and ranchers across the United States to send frozen beef samples to the Center for Human Nutrition Studies at Utah State University for untargeted metabolomics analysis. Additional beef samples were bought in grocery stores as well.

Meat Collection & Pulverization

Farmers were asked to submit a minimum of three meat samples from different animals to the research team to ensure biological replication and sufficient statistical power for metabolite analysis from the pooled group (grass-fed and grain-fed). Ribeye steaks (IMPS/NAMP Food Service Cut # 112) were selected as the beef cut for the analysis. Steaks were stored in a -40°C freezer until preparation time, at

which point, they were defrosted, ground up using a meat grinder, and stored in test tubes in a -80°C freezer. Next, they were pulverized using a mortar and pestle in liquid nitrogen, and the meat powder was weighed out into tubes and stored again in the -80°C freezer until metabolomics analysis.

Metabolomics Analysis

Sample preparation and analysis were carried out by Metabolon (Cary, NC) as described previously (Evans et al. 2014). Briefly, samples were weighed, and recovery standards were added prior to the first step in the extraction process for quality control purposes. To remove protein and to recover chemically diverse metabolites, proteins were precipitated with methanol under vigorous shaking for 2 min followed by centrifugation (15000 × *g*) at -4°C. The resulting extract was divided into five fractions: two for analysis by two separate reverse phase (RP)/UPLC-MS/MS methods with positive ion mode electrospray ionization (ESI), one for analysis by RP/UPLC-MS/MS with negative ion mode ESI, one for analysis by HILIC/UPLC-MS/MS with negative ion mode ESI, and one sample was reserved for backup. All methods utilized a Waters ACQUITY ultra-performance liquid chromatography (UPLC) and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35,000 mass resolution. The MS analysis alternated between MS and data-dependent MS^n scans using dynamic exclusion with a scan range covering 70-1000 m/z. Metabolites were identified by automated comparison of the ion features in the samples to a reference library of chemical standard entries that considered the retention time, molecular weight (m/z), preferred adducts, and in-source fragments as well as associated MS spectra to properly distinguish metabolites. Peaks were quantified using area-under-the-curve. A data normalization step was performed to correct for variation resulting from instrument inter-day tuning differences by setting the medians to equal one (1.00) and normalizing each data point proportionately (termed "block correction"). Data are expressed as arbitrary units (AU) comparing relative abundances between samples.

Statistical Analysis

Differences in metabolites in beef samples were determined using Welch's *t*-test with Benjamini-Hochberg adjusted *P*-values at 5% (False Discovery Rate; FDR < 0.05). Multivariate analyses such as Heatmaps and Volcano Plot were derived to determine the most important biochemicals that potentially discriminate grass-fed samples from grain-fed beef . Statistical analyses were performed in MetaboAnalyst 5.0 (**www.metaboanalyst.ca**). Bioactivities and potential health effects of annotated metabolites from the metabolomes was explored by using the FooDB and PubChem databases, while metabolic pathway identification used the Kyoto Encyclopedia of Genes and Genomes (KEGG).

Results and Discussion

A total of 75 ribeye steaks were analyzed for this study, 56 were characterized as grass-fed, and grass-finished and 19 were characterized as grain-finished based on farmer answers to questionnaires and/or package labeling. The untargeted metabolomics analysis of beef samples identified 182 amino acids, 44 carbohydrates, 39 cofactors and vitamins, 13 energy-associated metabolites, 372 lipids, 51 nucleotides, 2 partially characterized molecules, 51 peptides, 29 phytochemicals, 40 unknowns, 7 xenobiotics. Out of 802 profiled compounds, 191 were significantly different between the grass-fed and grain-fed beef groups (all, $p<0.05$). The Volcano plot (**Figure 1A**) indicates the directionality of the most discriminatory metabolites and found 44 metabolites that were >2.0-fold increased, in the grass-fed samples and 14 metabolites that were >2.0-fold increased in the grain-fed samples ($p<0.05$). Metabolites enhanced in grass-fed beef included long-chain polyunsaturated lipid metabolites such as linolenate (LA; 18:3n3), eicosapentaenoate (EPA; 20:5n3), various long-chain acyl carnitines, and phytochemicals such as methionine sulfone, catechol sulfate, dimethyl sulfone, 4-ethylphenylsulfate, and cinnamoylglycine. On the other hand, grain-fed samples were found to be enhanced in xenobiotics such as 2,8-quinolinediol sulfate (a potential anti-biotic residue), the phytochemicals trigonelline and piperidine (two alkaloids), and 4-hydroxy-nonenal-glutathione (a common marker of increased oxidative stress).

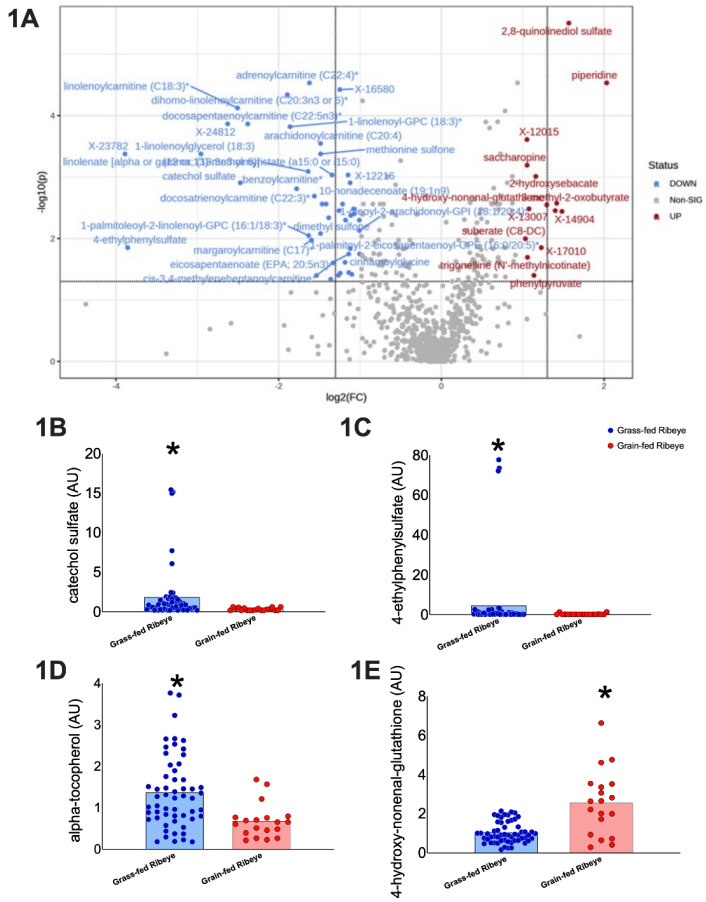

Figure 1.

Volcano Plot of Significantly Different Metabolites in Grain-fed Beef Compared to Grass-fed Beef (**1A**) found 44 metabolites were significantly lower in grain-fed compared to grass-fed samples (blue) and 14 metabolites were significantly higher (p<0.05) in the grain-fed samples compared to grass-fed samples (red). Catechol sulfate (**1B**), 4-ethylphenylsulfate (**1C**), and Vitamin E, alpha-tocopherol, (**1D**) were 5.5-fold, 14.5-fold, and 2.0-fold higher in grass-fed compared to grain-fed samples, respectively. However, 4-hydroxy-nonenal-glutathione (**1E**) was 2.5-fold higher in grain-fed beef compared to grass-fed beef. *Indicates a significant group difference (p<0.05). [AU, arbitrary units]

Phytochemicals

Phytochemicals are plant-derived secondary compounds that have been evaluated for their anti-oxidant and anti-inflammatory effects in both ruminants (Waghorn and McNabb 2003) and humans (Durazzo et al. 2019). When all 29 phytochemicals were summed together to produce a total phytochemical score, grass-fed beef had 1.7-fold higher levels of phytochemicals than grain-fed beef. It must be noted that

we found a 4.4-fold variation amongst individual farms, particularly those classified as grass-fed. Phytochemicals such as 4-ethylphenylsulfate, catechol sulfate, n-methylpipecolate, histidine betaine, and cinnamoylglycine were significantly more abundant in grass-fed samples (all, $p<0.05$). Hippurate is the glycine conjugate of benzoic acid and is metabolized into catechol sulfate and 4-ethylphenylsulfate. On average, catechol sulfate and 4-ethylphenyl sulfate were 5.5- and 14.5-fold higher in grass-fed compared to grain-fed beef **(Figure 1B, 1C)**. Dietary phenolic intake can be estimated with hippurate (Lees et al. 2013), and elevated levels measured in human blood plasma have been linked to a more diverse gut microbiome and better metabolic health in humans (Brial et al. 2021). Other phytochemicals such as fagomine, trigonelline, and piperidine were enhanced in the grain-fed samples. Trigonelline and piperidine are common alkaloids, with the latter having potentially toxic effects on livestock when consumed in high quantities (Green et al. 2012).

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Vitamins

Considering that meat is composed of animal cells that use vitamins and cofactors for metabolism, meat is known as a rich source of essential vitamins. Vitamins that showed little substantive mean differences included Vitamin B1, Vitamin B2, Vitamin B6, Vitamin C, dehydroascorbate, and choline, however, large variations were found amongst individual samples (up to 40-fold). Vitamin E, alpha-tocopherol, was 2-fold higher in grass-fed samples compared to grain-fed samples(**Figure 1D**). Vitamin B5, a pantothenate, was 1.2-fold higher in grain-fed beef compared to grass-fed beef. Nicotinamide, a form of Vitamin B3, was found to be 1.3-fold greater in grain-fed samples compared to grass-fed samples. This is explained by the observation that grains are typically higher in pantothenate and nicotinamide.

Animal Health Markers

4-hydroxy-nonenal-glutathione (4-HNE), a commonly studied marker of oxidative stress, was 2.5-fold upregulated in grain-fed beef compared to grass-fed beef samples, though considerable variation exists among samples (up to 38-fold), particularly in grain-fed samples (**Figure 1E**). 4-HNE is associated with metabolic dysfunction across mammalian species (Li et al. 2022; Vliet et al. 2022). Homocysteine, another marker of oxidative stress (Tyagi et al. 2005), as well as its biosynthetic precursor S-adenosylhomocysteine, were 1.3-fold higher in grain-fed beef compared to grass-fed beef; however, these compounds did not reach statistical significance and displayed considerable variation amongst individual samples. Pyrraline, an advanced glycation end-product was 1.9-fold higher in grass-fed beef compared to grain-fed beef, predominantly resulting from 4 grass-fed beef samples that were >2 standard deviations higher than other grass-fed beef samples.

Conclusion

Metabolomics analysis of grain-fed and grass-fed beef samples showed several distinct and previously unrecognized differences between the two types of production systems. We also found considerable variation within production systems, particularly in grass-fed beef. The source of that variation will have to be explored in future work. 44 metabolites were over 2-fold elevated in the grass-fed group, which showed enhanced phytochemical richness compared to the grain-fed group. 14 metabolites were 2-fold elevated in the grain-fed group including vitamins B3 and B5; however, grain-fed beef samples also had higher levels of 4-HNE potentially indicating increased oxidative stress. We found considerable complexity within the beef metabolome, and relative amounts of metabolites discovered here could provide new avenues to improve beef nutrient density and animal metabolic health by manipulating forages and concentrates fed to animals. It is currently unknown if the presence of these compounds in beef has an appreciable effect on human health, which must be explored in future human feeding trials.

Acknowledgements

Funding for this work was provided by USDA-2020-38640-31521, Bionutrient Institute, and Applegate LLC. We thank Shawna Marble, Dan Kittredge, Adie Prezenger, Amber Subers, and Amanda Pinelli at the Bionutrient Institute for project coordination .

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