npj | science of food

Article

Published in partnership with Beijing Technology and Business University

https://doi.org/10.1038/s41538-024-00315-5

Fatty acids and secondary metabolites can predict grass-finished beef and supplemental cattle feeds

Lucas Krusinski[1,2], Isabella C. F. Maciel[3], Stephan van Vliet[4], Muhammad Ahsin[4], Julianna Adams[1], Guanqi Lu[5], Chad A. Bitler[6], Jason E. Rowntree[3] & Jenifer I. Fenton[1]

Beef raised using rotational grazing practices on biodiverse pastures offers potential benefits to animal and environmental health and can improve the nutrient density of meat to favor human health. However, many cattle producers contend with the seasonal unavailability of fresh forage, necessitating the utilization of supplementary feeds or indoor feeding. The objective of this study was to profile secondary metabolites and fatty acids in grass-finished beef supplemented with different feeds (4.5 kg/head/day) and to explore the potential for grass-finished beef authentication. In this two-year study, steers (n = 115) were randomly allocated to one of four diets: 1) pastured/supplemented with hay (control group), 2) pastured/supplemented with baleage, 3) pastured/supplemented with soybean hulls, or 4) baleage/soybean hulls in confinement. Secondary metabolites and fatty acids were measured using UHPLC-MS/MS and GC-MS, respectively. Of the 94 measured metabolites, pyridoxine, alpha-tocopherol, hippuric acid, and gallic acid differed between diets ($p < 0.05$ for all). Based on random forest classification, beef from the pasture/hay, pasture/baleage, pasture/soybean hulls, and confinement baleage/soybean hulls groups could be identified with a predictive accuracy of 100%, 50%, 41%, and 97%, respectively. Although minimal significant differences were observed, our data indicate that certain supplemental feeds maintain favorable nutritional profiles of grass-finished beef. In addition, metabolomics can predict cattle on exclusively forage-based or feed-based diets with a high degree of certainty.

Given increasing concerns on the impact of beef production systems on environmental and human health[1], there is a need to study ways to improve production systems[2]. Some producers are working towards producing more sustainable and healthy beef by implementing rotational grazing practices (i.e., agroecological methods that more closely mimic natural herbivorous behaviors)[3]. Extensive agroecological systems also have the potential to benefit biodiversity[4]. When compared to beef finished in feedlots or on monoculture pastures, animal and human health are favored when cattle graze on phytochemically biodiverse pastures[5].

Previous work demonstrated that grass-finished beef (produced using rotational grazing methods) contains less fat, twice as much conjugated linoleic acid (CLA), more omega-3 (n-3) polyunsaturated fatty acids (PUFA), and a lower omega-6:omega-3 (n-6:n-3) ratio compared to grain-finished (feedlot) beef[6–8]. These key differences confirm that putting all beef under the same umbrella might be overly reductionist. Nutritionism (reductionist approaches to food) focuses on isolated nutrients in foods while ignoring the broader metabolic benefits[9]. It can also be applied to grass-finished beef, as one can argue that not all grass-finished beef is nutritionally equal. A recent nutritional survey of commercially available grass-finished beef found important differences between grass-finished beef coming from different producers, especially regarding the n-6:n-3 ratio, which ranged from 1.8:1 to 28.3:1, and in some cases exceeded typical feedlot beef values[10].

While most nutritional comparisons between beef from different production systems focused mostly on proteins, fatty acids (FA), and vitamins, less is known about secondary metabolites. Previous studies showed

[1]Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI, USA. [2]Schmid College of Science and Technology, Food Science Program, Chapman University, Orange, CA, USA. [3]Department of Animal Science, Michigan State University, East Lansing, MI, USA. [4]Center for Human Nutrition Studies, Department of Nutrition, Dietetics, and Food Sciences, Utah State University, Logan, UT, USA. [5]Department of Epidemiology and Biostatistics, Michigan State University, East Lansing, MI, USA. [6]Greenacres Foundation, Cincinnati, OH, USA. e-mail: imigjeni@msu.edu

that pasture-finishing can improve animal health and result in the transfer of bioactive metabolites with antioxidant and anti-inflammatory properties from feed to meat[11–13]. Such compounds are called phytochemicals and are produced by plants in response to herbivory, sunlight, soil conditions, and water availability[13,14].

Feeding fresh forages from diverse pastures to cattle usually results in the most beneficial beef nutritional profile[10,15,16]. However, some producers may rely on conserved forages to feed their animals on pasture when fresh grass is not available year-round[15,17], while others may opt for finishing on conserved forages in confinement, especially during winter months[18]. Dried (hay) or fermented (baleage) forages usually display reduced nutritional quality compared to fresh grass, including lower levels of antioxidants and phenolic compounds[16,19]. It was established that the concentration of secondary metabolites in forages is highly variable and depends on plant species and growing stages[20]. According to the American Grassfed Association (AGA), grass-finished cattle must consume only fresh grass and forages throughout their lifetime, with the inclusion of hay, baleage, and silages in case of inclement weather or low forage availability[21]. Although not allowed by the AGA, some producers may also feed soybean hulls (or other crop by-products) to their animals[21]. Soybean hulls are a fiber-rich by-product removed during soybean processing which has been fed to ruminants as an economical substitute for grain and hay[22].

Despite the potential benefits of finishing cattle on diverse pastures, less is known about the effects of supplementing grass-finished animals with conserved forages. Therefore, the goal of this study was to compare the nutritional profile (FA and secondary metabolites) of grass-finished beef supplemented with either hay, baleage, or soybean hulls using metabolomics, and to use the data to determine beef quality and explore the potential for the authentication of grass-finished beef. The four diets tested were 1) grazing supplemented with hay (GHAY, control group), 2) grazing supplemented with baleage (GBLG), 3) grazing supplemented with soybean hulls (GSH), and 4) baleage and soybean hulls in confinement (BLGSH).

Results

Nutritional and metabolic composition of the feeds and beef

The proximate composition and FA profile of the feed ingredients is shown in Table 1. The full phytochemical composition is reported in Supplementary Table 1. Main fatty acids quantified in beef are show in Table 2 (the full fatty acid profile is reported in Supplementary Tables 2 and 3). No differences were observed for total saturated FA (SFA) and monounsaturated FA (MUFA) ($p > 0.05$). Beef from GHAY contained significantly higher levels of PUFA ($p = 0.004$), especially n-3 PUFA ($p < 0.001$), including long-chain n-3 FA eicosapentaenoic acid (EPA) ($p < 0.001$), docosapentaenoic acid (DPA) ($p < 0.001$), and docosahexaenoic acid (DHA) ($p = 0.004$). Significant differences were also noted regarding the n-6:n-3 ratio with beef from GHAY displaying the lowest ratio, and beef from GSH displaying the highest ratio ($p < 0.001$). Table 3 shows 25 quantified metabolites in beef by diet (reported in mg per 100 g of beef). Pyridoxine concentrations were higher in beef from the GHAY group compared to the BLGSH group, while beef from GBLG and GSH did not differ compared to the other groups ($p = 0.042$). Alpha-tocopherol followed the same trend, with beef from GHAY containing more of this compound than beef from BLGSH and the other two groups not being significantly different compared to the rest ($p = 0.009$). Additionally, hippuric acid was almost two times higher in beef from GHAY compared to the other three groups. Beef from GHAY contained lower concentrations of gallic acid than beef from the other three dietary groups ($p = 0.006$). For all other quantified metabolites, no significant differences were observed between dietary groups ($p > 0.05$). The full list of identified beef metabolites (reported in arbitrary unit–AU) is reported in Supplementary Tables 4 and 5.

Data visualization of beef metabolites

Figure 1 shows group separation based on measured metabolites/factors. The principal component analysis (PCA) plot (Fig. 1a) shows 24.9% of data variance explained along principal component (PC) 1 with considerable overlap between groups. The random forest plot (Fig. 1b) displays the top compounds/factors leading to dietary group separation. Vitamin E and the n-6:n-3 ratio were the two most discriminating factors, followed by lipid oxidation values (TBARS), total n-3 PUFA, C22:5 n-3 (DPA), and C20:5 n-3 (EPA). Random forest classification (Fig. 1c) also demonstrated strong prediction between dietary groups, with an overall predictive accuracy of 73%. Beef fed the GHAY diet showed a 0% class error (100% predictive accuracy) followed by BLGSH with 97% predictive accuracy. The data indicate that beef fed exclusively grass (hay and pasture access) and beef

Table 1 | Nutritional composition of the diet ingredients

	GRASS	HAY	BLG	SH
Reported in % of total fatty acids				
C10:0	0.13	0.20	0.12	0.03
C12:0	0.47	0.72	0.40	0.03
C13:0	0.01	0.03	0.01	0.01
C14:0	0.49	0.80	0.53	0.18
C15:0	0.12	0.29	0.15	0.16
C16:0	16.53	28.50	18.87	14.47
C16:1 9c	0.24	0.36	0.25	0.23
C16:1 7c	1.34	1.53	1.52	0.11
C17:0	0.21	0.38	0.25	0.32
C18:0	1.62	3.02	1.86	4.35
C18:1 9c	2.19	3.03	2.34	13.15
C18:1 11c	0.59	0.84	0.65	2.52
C18:2 n-6 (LA)	13.67	14.20	13.32	48.39
C18:3 n-3 (ALA)	60.44	42.66	56.95	14.97
C20:0	0.61	1.15	1.03	0.43
C20:3 n-3	0.09	0.05	0.05	0.02
C22:0	0.61	1.03	0.92	0.35
C24:0	0.65	1.21	0.79	0.30
∑MUFA	4.36	5.75	4.76	16.01
∑PUFA	74.20	56.92	70.31	63.38
∑n-6	13.67	14.20	13.32	48.39
∑n-3	60.53	42.71	57.00	14.99
n-6:n-3 ratio	0.23	0.33	0.24	3.24
∑OCFA	0.33	0.70	0.41	0.48
∑SFA	21.44	37.33	24.93	20.62
∑FA	467.37	266.48	548.07	829.53
DM (%)	68.08	66.17	84.57	89.96
Ash[a]	9.11	8.34	6.99	4.55
CP[a]	15.03	9.13	12.41	9.24
NDF[a]	55.64	62.51	59.73	68.30
ADF[a]	31.35	34.46	30.69	52.40
Energy (cal/g)	4600.44	4434.40	4500.41	3774.07
Chlorophyll A (µg/g)	382.33	284.87	419.63	11.64
Chlorophyll B (µg/g)	424.69	275.93	225.20	30.15
Phenols (mg GAE/g)	4.93	6.15	12.04	1.11

Results reported as means.
GRASS diverse pasture mixture ($n = 15$), *HAY* dry hay ($n = 2$), *BLG* baleage ($n = 2$), *SH* soybean hulls ($n = 2$), *LA* linoleic acid, *ALA* alpha-linolenic acid, *∑MUFA* sum of monounsaturated fatty acids, *∑PUFA* sum of polyunsaturated fatty acids, *∑OCFA* sum of odd-chain fatty acids, *∑SFA* sum of saturated fatty acids, *∑FA* sum of all fatty acids, *DM* dry matter, *CP* crude protein, *NDF* neutral detergent fiber, *ADF* acid detergent fiber, *GAE* gallic acid equivalent.
[a]Reported as %DM.

Table 2 | Main fatty acids in beef by diet (mg/100 g beef)

	GHAY	GBLG	GSH	BLGSH	*p*-value
∑SFA	275.00 ± 48.50	275.00 ± 49.50	339.00 ± 50.00	356.00 ± 48.50	0.550
C12:0	0.57 ± 0.09	0.61 ± 0.09	0.74 ± 0.09	0.70 ± 0.09	0.534
C13:0	0.09 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.212
C14:0	12.40 ± 2.54	12.20 ± 2.61	15.30 ± 2.64	16.00 ± 2.54	0.647
C15:0	2.00 ± 0.31	2.15 ± 0.33	2.01 ± 0.33	2.02 ± 0.31	0.986
C16:0	163.00 ± 28.90	163.00 ± 29.50	206.00 ± 29.80	221.00 ± 28.90	0.427
C17:0	4.65 ± 0.93	4.90 ± 0.95	5.64 ± 0.96	6.01 ± 0.93	0.717
C18:0	87.00 ± 15.20	84.80 ± 15.50	101.40 ± 15.70	103.10 ± 15.20	0.770
∑MUFA	314.00 ± 47.10	313.00 ± 48.10	388.00 ± 48.70	372.00 ± 47.10	0.599
∑cMUFA	277.00 ± 43.20	272.00 ± 44.10	349.00 ± 44.60	342.00 ± 43.20	0.495
C16:1 9c	36.70 ± 5.68	35.90 ± 5.85	45.70 ± 5.93	44.20 ± 5.68	0.553
C18:1 9c	200.00 ± 34.30	197.00 ± 35.00	258.00 ± 35.30	255.00 ± 34.30	0.470
C20:1 9c	2.82 ± 0.19	2.66 ± 0.20	3.08 ± 0.20	2.92 ± 0.19	0.533
∑tMUFA	36.60 ± 4.92	41.00 ± 5.04	39.40 ± 5.10	29.80 ± 4.92	0.447
C16:1 9t	6.06^{a} ± 0.43	6.72^{a} ± 0.44	6.13^{a} ± 0.45	3.82^{b} ± 0.43	0.008
C18:1 9t	1.62 ± 0.34	2.41 ± 0.34	2.56 ± 0.34	2.52 ± 0.34	0.243
C18:1 11t	13.77 ± 2.83	12.56 ± 2.90	12.03 ± 2.94	5.63 ± 2.83	0.256
∑PUFA	98.30^{a} ± 3.54	72.80^{b} ± 3.68	76.70^{b} ± 3.74	73.70^{b} ± 3.54	0.004
C20:3 n-9	3.38^{a} ± 0.15	1.85^{b} ± 0.16	1.77^{b} ± 0.17	1.83^{b} ± 0.15	<0.001
∑n-6	47.60 ± 1.95	41.70 ± 2.03	48.40 ± 2.06	44.70 ± 1.95	0.162
C18:2 n-6	28.20 ± 1.28	25.50 ± 1.33	30.20 ± 1.36	27.40 ± 1.28	0.177
C22:4 n-6	5.05^{a} ± 0.21	2.69^{b} ± 0.22	3.36^{b} ± 0.22	3.29^{b} ± 0.21	<0.001
∑n-3	47.30^{a} ± 1.67	29.20^{b} ± 1.74	26.60^{b} ± 1.77	27.20^{b} ± 1.67	<0.001
C18:3 n-3	10.63^{a} ± 0.56	10.46^{a} ± 0.58	8.70^{b} ± 0.59	8.31^{b} ± 0.56	0.045
C20:3 n-3	0.89 ± 0.05	0.68 ± 0.05	0.74 ± 0.05	0.70 ± 0.05	0.059
C20:5 n-3	9.26^{a} ± 0.35	5.70^{b} ± 0.35	5.05^{b} ± 0.37	5.00^{b} ± 0.35	<0.001
C22:5 n-3	24.20^{a} ± 0.92	11.10^{b} ± 0.96	10.80^{b} ± 0.98	11.60^{b} ± 0.92	<0.001
C22:6 n-3	2.33^{a} ± 0.11	1.44^{b} ± 0.12	1.54^{b} ± 0.12	1.60^{b} ± 0.11	0.004
n-6:n-3 ratio	1.03^{c} ± 0.04	1.49^{b} ± 0.05	1.89^{a} ± 0.05	$1.70^{a,b}$ ± 0.04	<0.001
∑CLA	10.45 ± 0.76	8.38 ± 0.78	9.13 ± 0.79	7.11 ± 0.76	0.078
C18:2 9c,11t/9c,7t	6.26^{a} ± 0.56	$4.45^{a,b}$ ± 0.58	$4.74^{a,b}$ ± 0.59	3.05^{b} ± 0.56	0.027
∑FA	730.00 ± 99.10	701.00 ± 101.40	848.00 ± 102.50	840.00 ± 99.10	0.659

Results reported as mean ± standard error from the mean ($n = 115$). Different letters denote statistical significance at $p < 0.05$ according to the linear mixed model analysis.
GHAY beef out on pasture supplemented with hay, *GBLG* beef out on pasture supplemented with baleage, *GSH* beef out on pasture supplemented with soybean hulls, *BLGSH* beef fed baleage and supplemented with soybean hulls in confinement, ∑*SFA* sum of saturated fatty acids, ∑*MUFA* sum of monounsaturated fatty acids, ∑*cMUFA* sum of cis-monounsaturated fatty acids, ∑*tMUFA* sum of trans-monounsaturated fatty acids, ∑*PUFA* sum of polyunsaturated fatty acids, ∑*n-6* sum of omega-6 fatty acids, ∑*n-3* sum of omega-3 fatty acids, *n-6:n-3 ratio* ratio of omega-6 to omega-3 fatty acids, ∑*CLA* sum of conjugated linoleic acid isomers, ∑*FA* sum of all fatty acids.
Bolding refers to main fatty acid classes.

finished in confinement could be authenticated with a high degree of confidence. In contrast, the predictive accuracy for beef from GBLG and GSH was 50% and 41%, respectively.

To identify and visualize the relative abundance of the top fifty metabolites/phytochemicals in beef and the main metabolic pathways involved, a ranked heatmap and a pathway analysis plot were created (Fig. 2). Figure 2a displays the top fifty metabolites based on Euclidian distance measure and Ward clustering, differentiating between dietary groups, and including metabolite classes such as vitamins, phenols, non-protein amino acids, purines, fatty acyls, bile acids, and benzoic acids. To confirm enrichment of specific metabolite classes, a metabolic pathway analysis of all beef samples combined was conducted (Fig. 2b), which revealed the importance of specific pathways in the beef samples such as citrate (TCA) cycle, arginine biosynthesis, glyoxylate and dicarboxylate metabolism, arginine and proline metabolism, riboflavin metabolism, taurine metabolism, ketones biosynthesis and degradation, vitamin B_6 biosynthesis, retinol metabolism, and terpenoid backbone biosynthesis.

Discussion

Although minimal significant differences were observed between dietary groups, the eighty FA and ninety secondary metabolites reported showed that beef diets can be predicted with high levels of accuracy, indicating a high authentication potential. These results and the extensive list of compounds are important for ranchers and researchers interested in optimizing cattle finishing diets, as data indicate that conserved forages can be added to the diet of cattle on pasture without compromising the nutritional profile of beef. Additionally, the results show that phytochemicals from the feeds accumulate in the meat, indicating that beef might contribute to the dietary intake of secondary metabolites (albeit at lower concentrations than fruits and vegetables). Since all steers were of similar breed, the observed differences were likely due to the diet. Krusinski et al.[11] recently found differences in the secondary metabolite profile of beef finished on pasture or on grain in a feedlot, with most differences being observed for phenolics, vitamin E, and TCA cycle metabolites, which were enriched in grass-finished beef samples. Another metabolomics study in grass- and grain-finished bison also found

Table 3 | Quantified metabolites in beef by diet (mg/100 g beef)

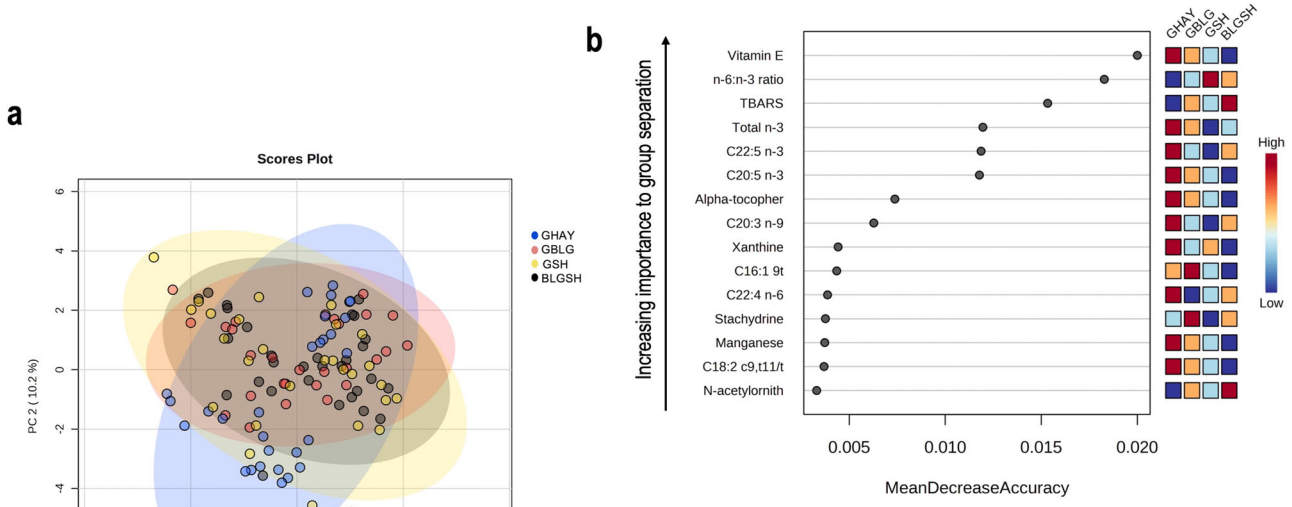
	GHAY	GBLG	GSH	BLGSH	*p*-value
B-vitamin metabolites					
Biotin	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.339
Niacin	1.94 ± 0.16	1.98 ± 0.16	2.18 ± 0.16	1.90 ± 0.16	0.641
Pyridoxine	0.02^{a} ± 0.00	$0.02^{a,b}$ ± 0.00	$0.02^{a,b}$ ± 0.00	0.01^{b} ± 0.00	0.042
Riboflavin	0.22 ± 0.04	0.23 ± 0.04	0.23 ± 0.04	0.18 ± 0.04	0.805
Thiamine	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.514
Phytochemicals/Secondary metabolites					
Alpha-tocopherol	0.86^{a} ± 0.07	$0.72^{a,b}$ ± 0.07	$0.57^{a,b}$ ± 0.07	0.41^{b} ± 0.07	0.009
Stachydrine	0.27 ± 0.02	0.33 ± 0.02	0.25 ± 0.02	0.27 ± 0.02	0.103
4-Ethylphenol	2.25 ± 0.31	1.96 ± 0.31	2.30 ± 0.32	1.89 ± 0.31	0.735
Betaine	0.57 ± 0.04	0.47 ± 0.04	0.58 ± 0.04	0.63 ± 0.04	0.092
Tyramine	8.99 ± 1.61	13.87 ± 1.67	12.48 ± 1.70	11.13 ± 1.61	0.267
Hercynine	0.15 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.589
Hippuric acid	20.50^{a} ± 2.17	12.40^{b} ± 2.21	10.50^{b} ± 2.24	11.20^{b} ± 2.17	0.042
Citric acid	120.5 ± 27.40	90.60 ± 28.00	134.90 ± 28.30	84.30 ± 27.40	0.555
Succinic acid	8.68 ± 2.73	14.18 ± 2.83	19.65 ± 2.88	13.07 ± 2.73	0.137
Fumaric acid	1.73 ± 0.31	1.16 ± 0.32	1.37 ± 0.32	0.78 ± 0.31	0.271
Chlorogenic acid	2.15 ± 2.61	9.00 ± 2.67	2.92 ± 2.70	3.32 ± 2.61	0.317
Caffeic acid	0.02 ± 0.02	0.00 ± 0.02	0.03 ± 0.02	0.01 ± 0.02	0.663
p-Coumaric acid	0.64 ± 0.10	0.39 ± 0.11	0.69 ± 0.11	0.81 ± 0.10	0.101
4-Hydroxybenzoic acid	0.26 ± 0.12	0.34 ± 0.12	0.43 ± 0.12	0.36 ± 0.12	0.775
Gallic acid	0.03^{b} ± 0.15	0.87^{a} ± 0.15	0.85^{a} ± 0.16	1.10^{a} ± 0.15	0.006
Ethyl gallate	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.635
Vanillic acid	0.02 ± 0.05	0.13 ± 0.05	0.08 ± 0.05	0.01 ± 0.05	0.332
D-Tartaric acid	0.67 ± 0.28	0.28 ± 0.29	1.31 ± 0.30	0.21 ± 0.28	0.106
Pyrocatechol sulfate	0.36 ± 0.05	0.37 ± 0.05	0.47 ± 0.05	0.39 ± 0.05	0.514
Coixol	1.58 ± 0.27	1.12 ± 0.28	1.27 ± 0.28	1.55 ± 0.27	0.596
∑Secondary metabolites	172.00 ± 26.10	151.00 ± 26.80	193.00 ± 27.10	134.00 ± 26.10	0.462

Results reported as mean ± standard error from the mean ($n = 115$). Different letters denote statistical significance at $p < 0.05$ according to the linear mixed model analysis.
GHAY beef out on pasture supplemented with hay, *GBLG* beef out on pasture supplemented with baleage, *GSH* beef out on pasture supplemented with soybean hulls, *BLGSH* beef fed baleage and supplemented with soybean hulls in confinement, ∑ sum.
Bolding refers to main metabolite classes.

that phenolics and energy metabolites were enriched in grass-fed animals, indicating improvements in animal health and higher amounts of potentially beneficial compounds for human health[12]. While pasture-finishing on fresh forages, as was the case in the aforementioned studies, indicate potential human and animal health benefits, the reality is that producers may need to finish on conserved forages when pasture is not available year-round[15,17].

Fresh grasses contain significant levels of phytochemicals that can potentially accumulate in ruminant meat[13]. Diverse pastures (composed of a wide variety of plant species) that are properly managed (e.g., not over-grazed) contain higher levels of alpha-tocopherol, carotenoids, chlorophyll, and phenols, which all have potential health benefits for humans and animals, compared to grain-based rations[6]. Even though haymaking usually results in a decrease in the PUFA and polyphenolic content of forages[23], hay may be the "gold standard" for grass-finished beef supplementation. This concept is reinforced by the 100% group predictive accuracy for the pasture supplemented with hay group (GHAY), while the other two groups out on pasture but supplemented with baleage (GBLG) and soybean hulls (GSH) were predicted with <50% accuracy. This indicates that supplementation with baleage or soybean hulls instead of hay may not be authenticated as 100% grass-finished beef. Additionally, we found that the grass and hay group had approximately two-fold higher levels of hippuric acid compared to the other groups. Hippuric acid is considered a good indicator of phenolic intake in mammals, including humans and livestock[11,12,24,25]. These data likely indicate that overall phenolic intake was highest in the group on pasture supplemented with hay.

Noteworthy is that baleage contained the highest levels of total secondary metabolites, particularly phenolic acids. This was observed from both the total phenolic content assay and metabolomics profiling of individual phenolic compounds. During the ensiling process, carbohydrates are converted into organic acids (and phenolic acids), while polyphenols (such as anthocyanins, flavonols, and flavanols) are degraded[26,27]. In the current study, gallic acid is an example of phenolic acid that was found in higher concentrations in beef supplemented with baleage compared to beef supplemented with hay. The feed data suggests that baleage and soybean hulls contain more gallic acid derivatives (e.g., epicatechin gallate) than hay. Soybean hulls were also investigated for their potential antioxidant properties[28], but results in previous studies indicate that soybean hulls may not significantly contribute to the phytochemical richness of beef[16]. Previous work showed that soybean hulls contain significant amounts of flavonols and other bioactive compounds, and that these concentrations depend on the plant's growth cycle[29]. The limited accumulation of bioactive compounds from soybean hulls in the meat may be related to their relatively high fiber content which can alter the rumen environment (i.e., biohydrogenation by bacteria and protozoa)[11,16,30]. However, there is currently only limited information about phytochemical metabolism in ruminants. While we found that baleage contained the highest levels of secondary metabolites, it is possible that our forage sample strategy did not adequately



c

	GHAY	GBLG	GSH	BLGSH	Class error
GHAY	30	0	0	0	0%
GBLG	3	14	10	1	50%
GSH	1	9	11	6	59%
BLGSH	0	0	1	29	3%
Predictive accuracy = 73%					

Fig. 1 | Metabolomic visualization of compounds in beef ($n = 115$) by diet. **a** Principal component analysis (PCA) showed some difference in metabolites between beef from different finishing diets, with 24.9% of the variance being attributed to principal component (PC) 1. **b** Random forest variable importance plot showed the top 15 factors capable of separating beef according to finishing diet. The y-axis represents compounds according to their importance to group separation (from top to bottom). The x-axis shows mean decrease accuracy, indicating the importance of the compound in predicting groups. **c** Random forest classification table shows an overall predictive accuracy of 73% between finishing diets. Data were log transformed, and both PCA and random forest analysis were produced using MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/). GHAY beef out on pasture supplemented with hay, GBLG beef out on pasture supplemented with baleage, GSH beef out on pasture supplemented with soybean hulls, BLGSH beef in confinement fed baleage and supplemented with soybean hulls, TBARS thiobarbituric acid reactive substances.

reflect or capture what was consumed by the grass and hay group (GHAY), which had higher levels of hippuric acid than the other groups, likely indicating the highest phenolic intake. Additionally, a broader number of polyphenols will have to be measured in future metabolomics analysis as the current analysis may have not captured certain polyphenols that appear to have contributed to the higher levels of hippuric acid in the GHAY group.

The importance of TCA cycle metabolites in grass-finished beef was highlighted in the pathway analysis. TCA cycle metabolites are most likely enriched in pasture-raised animals due to a combination of diet and exercise (e.g., grazing freely on pasture). Grass-finished animals display an oxidative phenotype, which is influenced by a diet high in long-chain PUFA (found in fresh grasses) and regular movement[12]. In the current study, B-vitamin metabolites in beef were affected by cattle diets, particularly levels of pyridoxine (vitamin B_6). Higher levels of vitamin B_6 were found in beef from animals out on pasture compared to the confinement group. Pyridoxine is mostly found in meat, fish, nuts, and some fruits and vegetables, and is associated with numerous health benefits in humans (physiological and neurological)[31]. Interestingly, others found higher levels of vitamin B_6 in confined animals compared to pasture-finished animals. The authors attributed these differences to higher levels of this vitamin in corn (usually found in feedlot diets)[12]. However, it is important to note that our confinement group was not a conventional feedlot group fed a high grain diet and did not contain corn.

Vitamin E, lipid peroxidation (TBARS) values, the n-6:n-3 ratio, and long-chain n-3 PUFA were the most discriminating factors capable of separating beef by dietary group according to the random forest analysis. These results align with previous findings[11,16]. Grass-finished beef usually contains up to three times more vitamin E than grain-finished (feedlot) beef[32–34], which is generally enough to protect meat from oxidation[35,36]. This is emphasized by the TBARS values (measure of lipid oxidation), which were lower in beef from the three groups out on fresh pasture compared to beef kept in confinement. In fact, vitamin E and TBARS followed strictly opposite patterns in the random forest classification plot. Higher amounts of vitamin E in beef usually correlate with lower TBARS values[37]. Supplementing grass-finished beef with hay or baleage was beneficial regarding these two factors compared to soybean hull supplementation[16]. Beef fed pasture-based diets also displayed higher n-3 PUFA concentrations compared to beef fed grain-diets[38]. Fresh grasses contain high levels of n-3 PUFA (mainly as alpha-linolenic acid–ALA, a precursor to the long-chain n-3 FA EPA, DPA, and docosahexaenoic acid–DHA), which increase total n-3 content and decrease the n-6:n-3 ratio in ruminants grazing such plants[6,38,39]. Haymaking and ensiling generally results in the oxidation of PUFA (coupled with higher palmitic acid concentrations)[40,41]. Additionally, soybean hulls contain higher levels of n-6 PUFA (~50% of total FAs)[16]. The results in the current study align with these findings, with the random forest plot showing beef from GHAY containing higher levels of n-3 PUFA, followed by beef from GBLG, GSH, and finally BLGSH. Logically, the opposite trend was observed for the n-6:n-3 ratio, which was previously identified as the most important discriminating factor to separate beef based on diet[11]. The n-6:n-3 ratio is considered by some as an important human health marker, with an ideal ratio being between 1:1 and 4:1[42–45]. A balanced ratio might be one of the most important dietary factors to prevent obesity-related diseases[44]. Others consider the n-6:n-3 ratio as far too simplistic to make nutritional claims, and recommend using the "Omega-3 Index" (taking into account long-chain n-3 PUFA such as EPA and DHA)

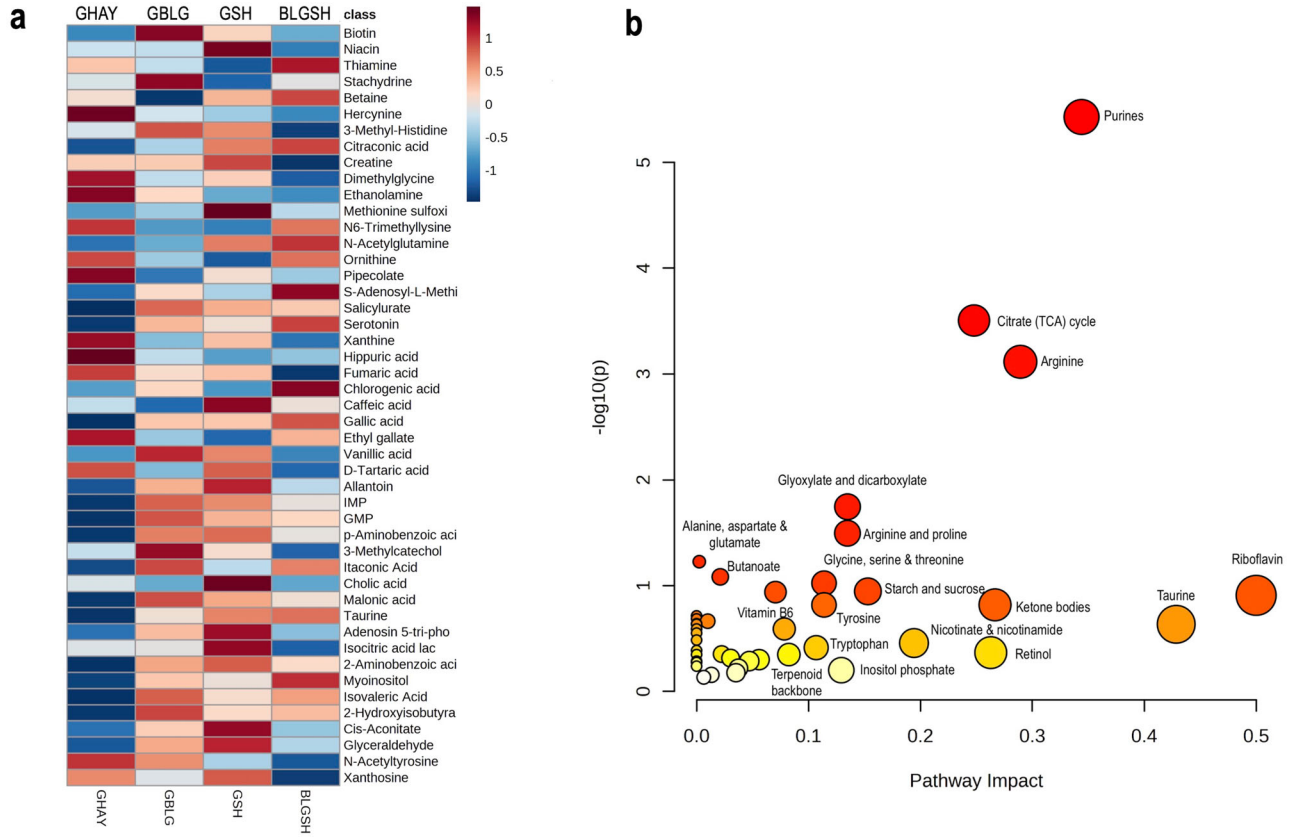


Fig. 2 | Distinctions observed in metabolites from varying classes in beef ($n = 115$). a Heatmap showing the top 50 compounds ranked by relative abundance (red being highest and blue being lowest) between beef from different finishing diets (averaged by groups). Data was log transformed and Euclidean distance measure and Ward clustering method were applied to produce the heatmap. **b** Metabolomics pathway analysis of all beef samples ($n = 115$) according to Kyoto Encyclopedia of Genes and Genomes (KEGG) *Bos taurus* library. A hypergeometric test was used for the pathway enrichment analysis, and topology analysis was conducted using the relative-betweenness centrality method. In the plot, the x-axis indicates the impact of the pathway, and the y-axis shows significant changes in the pathway according to identified compounds. Each node shows a matched pathway according to p-values from the enrichment analysis with different colors (varying from yellow to red) indicating different levels of significance. Different node sizes indicate varying impact values from the pathway topology analysis. Both heatmap and pathway plot were produced using MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/). GHAY, beef out on pasture supplemented with hay; GBLG, beef out on pasture supplemented with baleage; GSH, beef out on pasture supplemented with soybean hulls; BLGSH, beef in confinement fed baleage and supplemented with soybean hulls.

instead[46]. Nevertheless, the n-6:n-3 ratio has a strong beef authentication potential, as suggested by previous studies[11,47,48].

Metabolomics and lipidomics are powerful tools for the authentication and traceability of grass-finished products. As demonstrated in the present study, multiple variables (i.e., n-3 PUFA, the n-6:n-3 ratio, phytochemicals, TBARS) allowed for group separation with a degree of certainty especially for GHAY (100% predictive accuracy) and BLGSH (97% predictive accuracy). Pasture-raised ruminant products carry premium values and nutritional attributes of interest for health-conscious consumers, leading to a critical need for better authentication of such products[49]. Vitamin E, polyphenols, and FA were identified as factors that can be used for the authentication of products from grassland origins compared to grain-finished counterparts[47,50]. Indeed, this was confirmed by our findings given the 100% predictive accuracy of grass-finished beef supplemented with hay which fell to 50% and 41% when cattle were supplemented with baleage or soybean hulls on pasture, respectively. The current study is novel since the authentication potential of such analytical methods was pushed further to authenticate grass-finished beef based on supplemental feeds that may or may not be allowed under grass-fed/finished protocols. Even though third party organizations such as the American Grassfed Association have grass-fed protocols in place, their controls involve sending inspectors to farms to ensure said protocols were followed, but no empirical analyses are conducted to ensure the authenticity of products[21]. In France, Bleu-Blanc-Coeur is an association that touts sustainable, regenerative production systems that emphasize biodiversity and soil, plant, animal, and human health. Producers need to meet standards for their products to receive the Bleu-Blanc-Coeur label. Their technical guidelines emphasize the importance of FA, vitamins, energy metabolites, minerals, and secondary metabolites such as carotenoids, polyphenols, flavonoids, and tannins in beef. To make sure that producers follow their guidelines, they conduct regular analytical controls. One example is that the n-6:n-3 ratio in beef must be under 4:1[51]. Our current work shows potential for such programs in North America as well using the employed analytical methods.

Our work has limitations. While we do know the quantity of supplemental feeds provided to each group (4.5 kg/head/day), we did not record feed intake. The three groups out on pasture had access to a diverse mixture of forages containing five plant species, however, we did not determine to what extent steers consumed the supplemental feeds provided. Therefore, the exact diet of the animals cannot be established with certainty. The botanical composition of the pasture is known, but the proportions of each plant species is not, which makes it harder to draw connections between individual metabolites and plant species. Additionally, other studies hinted to the dose-dependent relationship between different feedstuffs and beef metabolites[11,52]. It is possible that the amounts of supplemental feeds fed to cattle in this study were too low to cause large metabolic differences. It is also important to note that the confinement group used in the present study was not a conventional feedlot group. Steers kept in feedlot are commonly fed high-energy, grain-intensive diets. Here, the confinement group was fed high quality baleage and supplemented with soybean hulls, which could explain the lack of differences observed between dietary groups, which is in

contrast to previous work from us and others[11,48]. In particular, our feedlot group had an n-6:n-3 ratio of 1.7:1. Finally, beef is a complex food matrix, and we cannot guarantee that all metabolites were extracted and identified. The field of food-omics is still in its early days, and we are confident that as the field progresses, more metabolites will be identified and quantified.

Metabolomics and lipidomics helped separate beef from different finishing diets as seen with the 100% predictive accuracy for the GHAY group and the 97% predictive accuracy for the BLGSH group. Identified compounds included n-3 PUFA, vitamins, polyphenols, and TCA cycle and energy metabolites with potential health benefits. Beef can potentially contribute additional health-promoting metabolites that are upcycled from plants otherwise not consumed by humans[13]. The tested supplemental feeds appear to be noteworthy alternatives to grains, and do not compete with human consumption. European Alpine production systems, hyped for the nutritional profile of their ruminant products, graze their animals during spring and summer months, and keep their animals indoors with conserved forages in the winter[53]. Even animals kept in confinement and fed conserved forages produced beef rich in antioxidant secondary metabolites (an option that could be explored to reduce reliance on grains in feedlot diets). There are nutritional tradeoffs with each diet/supplemental feed, but the data published here can be used for the authentication of grass-finished beef. It was confirmed that the n-6:n-3 ratio, vitamin E, long-chain n-3 PUFA, and some phytochemicals are crucial factors for the authentication of products of grassland origins.

Even if only slight differences between beef from varying dietary groups were observed, the dataset still allowed separation of beef from different finishing diets with high levels of confidence. These results are important for ranchers and researchers interested in optimizing cattle finishing diets, meat nutrient density, and authentication. Null results are part of the research process and are crucial to informing the next research questions to be tested[54]. Future studies are needed to understand the effects of the consumption of grass-finished beef supplemented with different feeds on human health. Additional supplemental feeds such as distiller's grains should be tested to potentially identify causes of variations in the nutritional profile of commercial grass-finished beef as observed previously. The effects of carcass fatness and marbling score on the accuracy of grass-finished beef authentication should also be considered.

Methods

The utilization of animals and the protocol employed have been granted approval by the Institutional Animal Care and Use Committee at Michigan State University (IACUC #201800155). All experiments were performed in accordance with relevant guidelines and regulations. The manuscript was cross-checked with the ARRIVE Essential 10 checklist (Supplementary File – The ARRIVE Essential 10).

Experimental design, animals, and diets

This study took place over two years (2020 and 2021) in Hickory Corners, Michigan (latitude: 42°24′38″ N, longitude: 85°22′45″ W, elevation: 282 m) at the Michigan State University Kellogg Biological Station (KBS). Each year, sixty steers were randomly assigned to one of four dietary groups: the control group which was grass supplemented with hay (GHAY), grass supplemented with baleage (GBLG), grass supplemented with soybean hulls (GSH), or a diet consisting of baleage and soybean hulls in feedlot (BLGSH). For each diet, three groups were created, with each group comprising five animals (three replicates per diet and a total of 15 animals per diet) in each year. The allocation of animals to these three groups within each diet was done randomly after stratification. Each replication (paddock) had four acres split into one-acre sub-paddocks. Sub-paddocks were strip grazed with Monday, Wednesday, Friday movement. Each replication/paddock contained five animals. Since the study was conducted in Michigan (Midwest, USA), there is no fresh grass available year-round, especially in the winter, and hay is relied upon heavily in that region to meet animal nutritional requirements[17]. The AGA allows the use of hay when fresh forages are not available[21]. Therefore, the GHAY group was considered the control group in this study. Each year in April, sixty Simmental-Angus influenced feeder cattle with an average weight of 387 kg (±47 kg) were purchased from the same Oklahoma, USA producer and transported to KBS. Upon arrival at KBS, the initial weights of the steers were collected, and animals were randomly stratified by weight, and allocated to their diets. Random allocation of animals to treatments was done by the farm staff, and the researchers were unaware of the groups until the final statistical analysis. Steers assigned to the three grass-based diets were allowed to graze with unrestricted access to a diverse pasture mixture (GRASS) and were given 4.5 kg of supplemental feed (dry matter, DM) per head per day. The steers allocated to the confinement group had *ad libitum* access to baleage (BLG) and were given 4.5 kg of soybean hulls (SH) per head per day. The GRASS was a mixture of five species: alfalfa (*Medicago sativa*), red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.), orchard grass (*Dactylis glomerata* L.) and endophyte-free tall fescue (*Festuca arundinacea*). The dry hay (HAY) was a mixture of alfalfa (*Medicago sativa*), orchard grass (*Dactylis glomerata* L.), and tall fescue (*Festuca arundinacea*). BLG was composed of alfalfa (*Medicago sativa*) and orchard grass (*Dactylis glomerata* L.). A fenced paddock was designated for each subgroup within each of the grass-containing diets. Each diet was allocated three paddocks, and five animals were kept within each paddock. To give the pasture time to rest and regrow, each paddock was divided into sub-paddocks. Steers were rotated to a different sub-paddock with fresh parcels of grass within their paddock three times per week using portable fencing. The fifteen steers following the BLGSH diet were managed as feedlot cattle and divided among three pens, with five animals in each pen. During the first year of the study, one animal died and in the second year, the slaughterhouse misplaced two carcasses, and two samples were not suitable for analysis. This resulted in a total of 115 animals for the entire study ($n = 115$).

Sample collection and preparation

Feed samples were profiled for the length of the study, and results were previously reported[16]. Pasture samples (GRASS) were collected from grazing areas and supplemental feeds were collected from feeders. Each year, the sample collection occurred in late July (July 23, 2020, and July 26, 2021). There were no samples collected between the months of April and July of 2020 due to restrictions following COVID-19. For reasons of consistency, the sample collection took place during the same period for the 2021 collection. Immediately before animals had access to grazing areas, GRASS samples were collected in each sub-paddock ($n = 15$ in total). To collect samples of GRASS, hand grass clippers were used to cut three random 0.25 m^2 quadrants to a 5 cm stubble. For HAY, BLG, and SH, samples were collected prior to being distributed to steers ($n = 2$ for each supplemental feed). For proximate analysis, wet feed weights were recorded prior to samples drying in a forced-air oven for 72 h (55 °C). Dried samples were processed and ground using a Wiley mill through a 1 mm screen (Arthur H. Thomas, Philadelphia, PA, USA). For phytochemical and FA analysis, a 30 g sub sample was packed in a Whirl-Pak bag and frozen at −20 °C right after collection. To ensure that feed samples were representative, the contents of the bag were mixed, and 10 g from each replicate was taken before being combined. Samples were stored at −20 °C for the duration of the trial prior to being stored at −80 °C after they were brought back to the laboratory. Before conducting further analysis, samples were freeze-dried in a freeze dryer (Harvest Right, North Salt Lake, UT, USA) for 18.5 h. The freeze-dried samples were ground in a Wiley mill through a 1 mm screen with dry ice.

For meat samples, steers were slaughtered each year in November at 18-20 months of age. Slaughter took place in a United States Department of Agriculture (USDA)-regulated facility in the presence of an accredited inspector. Animals were stunned using a compressed air desensitization pistol before being slaughtered by rapid exsanguination. Body performance and carcass characteristics were previously reported[16]. Meat samples measuring approximately 7.5 cm to 10 cm in length were collected from the left side *longissimus lumborum* (between the 11th and 13th rib). One steak per carcass was cut into 1 × 1 cm cubes. The cubes were rapidly frozen using liquid nitrogen and placed into Whirl-Pak bags. Samples were stored at

−80 °C until analysis. Beef and feed samples underwent FA analysis at the end of each year (November 2020 and 2021). Samples were flushed with nitrogen gas and stored at −80 °C to limit oxidation and sample degradation, as the recommended freezing temperature to protect meat from lipid oxidation is −40 °C or lower[55]. Protection from oxygen is also crucial to preserve samples before and during analysis[56]. Beef and feed samples underwent metabolomics analysis between March and April 2023. The beef tissue and feed samples used for metabolomics were the same as for the FA analysis. To prevent sample degradation, all samples were flushed with nitrogen gas and stored at −80 °C. At −70 °C, all diffusion-limited reactions (including enzymatic activity, especially polyphenol oxidase) cannot occur due to reduced molecular mobility of the system[57].

Feed proximate analysis

Feed proximate analysis was conducted using methods previously described[58]. Samples were dried in a forced-air oven (105 °C for 8 h). For ash content, samples were oxidized in a muffle furnace (500 °C for 6 h). To measure neutral detergent fiber (NDF), the protocol described by Mertens[59] was performed (which included the use of amylase and sodium sulfate). To determine acid detergent fiber (ADF) content, the methods described in AOAC[60] were used. Methods described previously[61] were used to measure crude protein (CP). Finally, a bomb calorimeter was used to measure gross energy.

Feed chlorophyll and total phenols analysis

The determination of Chlorophyll A and B was conducted using the methodology previously outlined[62]. Briefly, a 2 g sample of freeze-dried forage was introduced into a solution of 70% aqueous acetone. The solution was shaken for 30 min and centrifuged for 20 min at 4 °C and 840 × *g* (2500 rpm in a Sorvall Legend RT+ centrifuge, Thermo Fisher, Waltham, MA, USA). The top layer was transferred into a new tube, and the extraction process was repeated twice. Chlorophyll A and B were measured in cuvettes using a UV-Vis Double Beam Spectrophotometer (VWR, Radnor, PA, USA). Readings were taken at wavelengths 663 and 646 nm and were applied in Eqs. (1) and (2):

$$\text{Chlorophyll A}\left(C_a\right) = 12.21A_{663} - 2.81A_{646} \quad (1)$$

$$\text{Chlorophyll B}\left(C_b\right) = 20.13A_{646} - 5.03A_{663} \quad (2)$$

where A_x is the absorbance reading at a certain wavelength.

A modified protocol[63] was used to extract phenolic compounds. Briefly, a 2 g lyophilized and ground feed sample was introduced into 20 mL of methanol:distilled water:acetic acid (70:28:2, *v*/*v*/*v*). The solution was shaken for 30 min and centrifuged for 20 min at 4 °C and 840 × g (2500 rpm). The top layer was transferred into a new tube. An additional 20 mL of acetone:distilled water:acetic acid (70:28:2, *v*/*v*/*v*) was added to the initial tube. Samples were then shaken for 10 min and centrifuged for 15 min (4 °C, 840 × *g*, 2500 rpm). The two supernatants were combined and stored at 4 °C. The assessment of the total phenolic content was conducted using the Folin-Ciocalteu assay, which was adapted from Singleton and Rossi[64]. Briefly, 1 mg/mL of a gallic acid solution in methanol was used to generate the standard curve. Concentrations ranging from 1 mg/mL to 0.002 mg/mL were obtained by performing a two-factor serial dilution. Then, 100 µL of Folin-Ciocalteu reagent and 800 µL of 5% sodium bicarbonate were introduced to both the standard curve and 100 µL of supernatant. Both the standard curve and samples were heated for 30 min at 40 °C. Samples were cooled to room temperature (22–25 °C) prior to being plated in triplicates in a 96-well plate. Samples were then scanned at a wavelength of 765 nm and compared to the gallic acid standard curve. Total phenolic content was reported as mg of gallic acid equivalents (GAE)/g of feed.

Fatty acid analysis of feed and meat

Unless otherwise specified, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were used within 20 weeks. The analysis was conducted according to previously published methods[16,38,65]. Microwave-assisted extraction was conducted using a CEM Mars 6 microwave (CEM Corp., Matthews, NC, USA)[10]. For the extraction, 8 mL of 4:1 (*v*/*v*) ethyl acetate:methanol solution with 0.1% BHT was added to microwave tubes followed by 400 mg of minced meat or ground feed samples. The microwave settings were set to 55 °C for 15 min with initial ramp of 2 min at 400 W. Samples were then filtered into tubes containing 3.5 mL of HPLC water and then centrifuged at 840 × *g* (2500 rpm) for 6 min. The upper layer was extracted and dried under nitrogen. To bring the concentration of each sample to 20 mg of oil/mL, the oil was resuspended using a 4:1 (*v*/*v*) dichloromethane:methanol solution with 0.1% BHT.

Fatty acid methyl esters (FAME) were created using modified methods[66]. Briefly, 2 mg samples were resuspended with toluene and 20 µg of an internal standard (methyl 12-tridecenoate, U-35M, Nu-Chek Prep, Elysian, MN, USA). Next, 2 mL of 0.5 N anhydrous potassium methoxide was introduced to the samples prior to a 10 min heating period at 50 °C. Then, 3 mL of methanolic HCl (5%) was introduced to the samples prior to a 10 min heating period at 80 °C. Samples were allowed to cool to room temperature before 2 mL of HPLC water and 2 mL of hexane were added. Samples were then centrifuged at 840 × *g* (2500 rpm) for 5 min. The upper layer of the samples was moved into a fresh tube and dried under nitrogen, leaving behind the FAME. Finally, FAME were resuspended in 1 mL of isooctane to reach a final concentration of 2 mg/mL. Samples were pipetted into gas chromatography-mass spectrometry (GC-MS) vials with glass inserts.

To quantify FAME, the PerkinElmer (Waltham, MA, USA) 680/600S GC-MS set to electron impact mode (70 eV) equipped with an Agilent Technologies (Santa Clara, CA, USA) HP-88 column (100 m, 0.25 mm ID, 0.2 µM film thickness) was used. One µL of feed sample was injected in the GC with the temperature set at 250 °C. One µL of meat sample was injected twice (20:1 split) at two different GC temperatures, 175 °C and 150 °C. The temperature settings for both feed and meat samples were as follows: initial temperature at 80 °C for 4 min; ramp 13 °C/min to 175 °C; hold 27 min; ramp 4 °C/min to 215 °C; hold 35 min, and then an initial temperature at 80 °C for 4 min; ramp 13 °C/min to 150 °C; hold 47 min; ramp 4 °C/min to 215 °C; hold 35 min. Additionally, for the meat samples, a third injection was conducted following a splitless mode (0.75 min splitless hold time, 40 mL/min flow exiting the vent). The GC-MS protocol used was modified from previously outlined methods[67]. The carrier gas used was helium, with a flow rate of 1 mL/min. Full scan mode (mass range of m/z 70-400 amu) was used on the MS for data acquisition and the MS transfer line and ion source were maintained at a temperature of 180 °C.

To identify FAME, MassLynx V4.1 SCN 714 (Water Corp., Milford, MA, USA) was utilized. Retention time was used to identify FA. Electron impact (EI) mass fragmentation was conducted in comparison with the reference standard containing the Supelco 37 Component FAME Mix with mead acid, docosatetraenoic acid, n-3 DPA, n-6 DPA, and palmitelaidic acid purchased from Cayman Chemical (Ann Arbor, MI, USA). CLA isomers were identified through a CLA reference standard UC-59M (Nu-Chek Prep, Elysian, MN, USA). EI mass fragmentation and elution order were used to identify FA not included in the reference standard[67]. Quantification of FA was conducted using a standard curve that incorporated both reference and internal standards. To determine the concentration of each FAME, the peak area of the internal standard and peak area of the analyte was compared to the standard curve. Notably, C18:14t and C18:15t were below detectable limit and C18:2 9c,12t and C18:2 9t,12c could not be distinguished from the C12:2 11t,15c peak. Eicosatetraenoic acid (C20:4 n-3) was absent from our reference standard and therefore not included in our report.

Metabolomics

Using a Harvest Right Home Freeze Dryer (Harvest Right, North Salt Lake, UT, USA), feed samples were freeze-dried for 18.5 h and ground through a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA) equipped with a 1 mm screen with dry ice[6]. Using a mortar and pestle, beef samples were pulverized on dry ice. The following was ordered from Sigma-Aldrich (St.

Louis, MO, USA): UHP-LC-MS-grade acetonitrile, methanol, DMSO, formic acid, and water (Supelco LiChrosolv®), and all chemicals were used within 4 weeks of purchase. The QReSS™ internal standards kit that contained a mixture of isotopically labeled metabolites was ordered from Cambridge Isotope Laboratories (Tewksbury, MA, USA). Lastly, purified external standards of compounds were ordered from Sigma-Aldrich (St. Louis, MO, USA) and/or Cayman Chemical (Ann Arbor, MI, USA).

Procedures described previously were followed for this analysis[11,12]. In brief, 200 mg pulverized beef samples and 50 mg feed samples were mixed with 1000 µL and 500 µL of methanol, respectively. Then, 10 µL of QReSS™ internal standard was introduced to the samples. Using a QIAGEN TissueLyser II operated with two 5 mm glass beads (QIAGEN Sciences, Germantown, MD, USA), proteins were precipitated under vigorous shaking for 10 min at 20 Hz. Next, samples were subjected to a 1 h protein freeze-out (at −20 °C) prior to being centrifuged at 23,000 × *g* for 10 min at 4 °C (15,636 rpm in an Eppendorf Centrifuge 5424R, Eppendorf, Hamburg, Germany). The resulting supernatant was extracted and moved to a fresh set of tubes. Two mL of water with 1% formic acid (*v*/*v*) was used to dilute the beef samples and 1 mL of the same solution was used to dilute feed samples. For solid phase extraction (SPE), Strata C18-E cartridges (Phenomenex, Torrance, CA, USA) were used. To activate cartridges, they were treated with 1 mL of methanol with 1% formic acid and washed with 1 mL of water with 1% formic acid. Then, samples were passed through the cartridges, and beef samples were washed with 2 mL of water with 1% formic acid while feed samples were washed with 1.2 mL of water with 1% formic acid. Both beef and feed samples were eluted with 1.2 mL of methanol in 0.1% formic acid. Samples were evaporated under nitrogen gas prior to being reconstituted with 100 µL and 200 µL, respectively, of methanol in 0.1% formic acid in 1.5 mL LC-MS amber vials with 250 µL glass inserts (Agilent, Santa Clara, CA, USA).

Compounds were identified by monitoring precursor ion/product ion pair using multiple reaction monitoring (MRM) via ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). A SCIEX Hybrid Triple Quad™ 7500 (Framingham, MA, USA) with a front-end Shimadzu Nexera LC-40 Series (Kyoto, Japan) liquid chromatography system was used. Samples were kept at 10 °C in an auto-sampler, and separation of compounds was conducted at 30 °C using a reverse phase Kinetex F5 100Å column (2.1 mm × 150 mm, 1.7 µM) from Phenomenex (Torrance, CA, USA) with binary mobile phases of water (A) and acetonitrile (B), both containing 0.1% formic acid (*v*/*v*). Samples were analyzed in both negative and positive electrospray ionization mode. Exact equipment parameters for the negative and positive modes were described previously[11]. For both modes, an unscheduled method was used to determine the presence of compounds in the matrix and establish their retention time for the scheduled method. A pooled matrix sample (sample generated by taking a small volume from samples from different experimental conditions, one sample/pen/year, to form a representative sample), a double-blank (100% methanol), and a mixture of purified standards of target compounds were run for quality control and to check for instrument stability. The pooled sample was created with pulverized beef samples before extraction. To generate data, each individual animal sample was run separately. For both modes, the cycling time for the scheduled method was set to 1000 msec. The dwell time was adjusted from 3 to 250 msec depending on the number of MRM triggered. For every 15 samples, double-blank (100% methanol) and blank internal standard samples (methanol spiked with QReSS™ isotopically labeled internal standards) were run for quality control purposes.

Chromatographic data were analyzed using Analyst 3.1 software (AB Sciex, Framingham, MA, USA). Peak integration was performed using the area under the curve and normalization was conducted using QReSS™ isotopically labeled internal standards. This approach was used to account for any lost material in the sample preparation process. To quantify unlabeled external standard mixes (in mg/100 g), they were run parallel to the samples with known concentrations of the various metabolites with relevant nutritive/metabolic value for which standards were available. In cases where compounds lacked nutritive/metabolic value, or for which the standard was not run concurrently, data were expressed as arbitrary units (AU).

Beef vitamin E and mineral analysis

The vitamin E analysis protocol outlined by Rettenmaier and Schüep[68] was followed, as described previously[16]. Briefly, 1 g of beef was homogenized in 5 mL of water prior to being frozen. For the extraction process, samples were thawed, and a measured aliquot was pipetted out. For protein precipitation, ethanol was introduced, and hexane was used for the extraction of fat-soluble vitamins. Following centrifugation, a portion of the hexane layer was extracted and evaporated. The residue obtained was solubilized in the chromatographic mobile phase and transferred to vials. The chromatography analysis was performed using a Waters Acquity system and Water Empower Pro Chromatography Manager software (Water Corp., Milford, MA, USA). An isocratic elution was conducted utilizing the following mobile phase: acetonitrile:methylene chloride:methanol (70:20:10, *v*/*v*/*v*) and a Symmetry C18, 1.7 µm, 2.1 × 50 mm analytical column (Waters Corp., Milford, MA, USA). The flow rate was set at 0.5 mL/min and a detection was conducted using a UV absorption of 295 nm.

Methods for mineral analysis were performed utilizing the previously described procedure[38,69]. In brief, an Agilent 7900 Inductivity Coupled Plasma–Mass Spectrometer (ICP-MS) (Agilent Technologies Inc., Santa Clara, CA, USA) was used, and a six-point calibration curve and standards of bovine liver and mussels (National Institute of Standards and Technology, Gaithersburg, MD, USA) were used as controls.

Thiobarbituric acid reactive substances (TBARS)

For this analysis, the TBARS assay for food and beverages from Oxford Biomedical Research (Oxford, MI, USA) adapted for a 96-well plate reader was used as described previously[16]. Initially, an eight-point standard curve was generated using a serial dilution, ranging from 0 (containing only HPLC water) to 3 g/L malondialdehyde (MDA) using the MDA stock solution provided in the kit. Next, 500 mg of minced beef sample was combined with 5 mL of HPLC water and homogenized to obtain a smooth solution. Then, 250 µL of sample solution and 250 µL of the indicator solution (thiobarbituric acid and acid solution) were mixed in a microcentrifuge tube. The indicator solution was also added to the standard curve. Both the samples and the curve were allowed to sit for 60 min to allow the reaction to occur. Afterwards, samples were centrifuged at room temperature for 5 min at 11,655 × *g* (11,000 rpm in a Sorvall Legend Micro 21 microcentrifuge, Thermo Fisher, Waltham, MA, USA). The aqueous layer was extracted and plated in duplicate next to the standard curve on the 96-well plate. The absorbance was measured at 532 nm on a Bio-Tek Synergy HT spectrophotometer (Bio-Tek Instruments Inc., Winooski, VT, USA).

Statistical analysis

To determine the number of animals to include for each treatment, a power analysis was performed as follows: $\alpha = 0.05$, $\beta = 0.80$, and coefficient of variation = 0.10. To detect a 10% difference in outcome variables, fifteen heads per treatment per year were needed.

To assess the impact of diet on quantified compounds, a linear mixed model analysis was run using RStudio (R version 4.3.1) (R Core Team, Vienna, Austria). The model included the fixed effects of diet, year, and their interaction, while random effects consisted of pen nested within diet. The experimental unit was defined as each pen. Post hoc comparison was conducted using Tukey's adjustment, and significance was established at $p < 0.05$. The alpha level for all analyses was 0.05 and the p-value for multiple comparisons was corrected by Dunn–Šidák correction. All tests were two-tailed. The data adhered to the normality and equal variance assumptions of the model (residuals of the model were normally distributed as checked with *qqplot* in RStudio). Results are represented as mean ± standard error across mean (SEM). Significant Diet:Year interactions are also reported in Supplementary Tables 3 and 5.

Statistical visualization of the metabolomics data was performed using MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/MetaboAnalyst/). To visualize differences between groups and determine the top compounds capable of group separation, unsupervised PCA and random forest analysis were conducted as described previously[11,12,70]. Random forest was chosen as it was described as a desirable post-hoc method that can enhance interpretation of metabolomics data thanks to its high expressive power and fidelity[71]. It also allows for direct comparison with studies that used similar methods[11,12,48]. For random forest analysis, the number of trees was 500, the number of predictors (estimators) was seven, and the randomness feature was turned on. No validation dataset was needed as out-of-bag (OOB) data was used to evaluate the quality of the model and prevent overfitting. A ranked heatmap was generated using the top fifty compounds, showing relative abundance, and averaged by group (Euclidean distance measure and Ward clustering). Pathway analysis for cow (*Bos taurus*) was performed using metabolite names identified in all beef samples, and main metabolic pathways were identified using the Kyoto Encyclopedia of Genes and Genomes (KEGG). A hypergeometric test was used, and topology analysis was conducted using the relative-betweenness centrality method. Secondary metabolites data were normalized to mass and log transformed.

Data availability

All data generated and/or analyzed during the current study are included within the article and its supplementary files. Additional data files can be provided by the corresponding author upon reasonable request.

Received: 7 February 2024; Accepted: 24 September 2024;
Published online: 04 October 2024

References

1. Willett, W. et al. Food in the Anthropocene: The EAT–Lancet Commission on healthy diets from sustainable food systems. *Lancet* **393**, 447–492 (2019).
2. Godfray, H. C. J. et al. Meat consumption, health, and the environment. *Science* **361**, eaam5324 (2018).
3. Spratt, E. et al. Accelerating regenerative grazing to tackle farm, environmental, and societal challenges in the upper Midwest. *J. Soil Water Conserv.* **76**, 15A–23A (2021).
4. Cozim-Melges, F. et al. Farming practices to enhance biodiversity across biomes: A systematic review. *npj Biodivers.* **3**, 1 (2024).
5. Provenza, F. D., Kronberg, S. L. & Gregorini, P. Is grassfed meat and dairy better for human and environmental health? *Front. Nutr.* **6**, 1–13 (2019).
6. Krusinski, L. et al. Evaluation of fatty acid and antioxidant variation in a complex pasture system as compared to standard cattle feed in the Great Lakes Region. *Front. Sustain. Food Syst.* **6**, 945080 (2022).
7. Van Elswyk, M. E. & McNeill, S. H. Impact of grass/forage feeding versus grain finishing on beef nutrients and sensory quality: The U.S. experience. *Meat Sci.* **96**, 535–540 (2014).
8. Alfaia, C. P. M. et al. Effect of the feeding system on intramuscular fatty acids and conjugated linoleic acid isomers of beef cattle, with emphasis on their nutritional value and discriminatory ability. *Food Chem.* **114**, 939–946 (2009).
9. Leroy, F., Beal, T., Gregorini, P., McAuliffe, G. A. & van Vliet, S. Nutritionism in a food policy context: The case of 'animal protein. *Anim. Prod. Sci.* **62**, 712–720 (2022).
10. Bronkema, S. M. et al. A nutritional survey of commercially available grass-finished beef. *Meat Muscle Biol.* **3**, 116–126 (2019).
11. Krusinski, L. et al. Measuring the phytochemical richness of meat: Effects of grass/grain finishing systems and grapeseed extract supplementation on the fatty acid and phytochemical content of beef. *Foods* **12**, 3547 (2023).
12. van Vliet, S. et al. Pasture-finishing of bison improves animal metabolic health and potential health-promoting compounds in meat. *J. Anim. Sci. Biotechnol.* **14**, 49 (2023).
13. van Vliet, S., Provenza, F. D. & Kronberg, S. L. Health-promoting phytonutrients are higher in grass-fed meat and milk. *Front. Sustain. Food Syst.* **4**, 1–20 (2021).
14. Serra, V., Salvatori, G. & Pastorelli, G. Dietary polyphenol supplementation in food producing animals: Effects on the quality of derived products. *Animals* **11**, 401 (2021).
15. Krusinski, L., Sergin, S., Jambunathan, V., Rowntree, J. E. & Fenton, J. I. Attention to the details: How variations in U.S. grass-fed cattle-feed supplementation and finishing date influence human health. *Front. Sustain. Food Syst.* **6**, 851494 (2022).
16. Krusinski, L. et al. Effects of hay, baleage, and soybean hulls waste used as supplemental feeds on the nutritional profile of grass-finished beef. *Foods* **11**, 3856 (2022).
17. Gwin, L. Scaling-up sustainable livestock production: Innovation and challenges for grass-fed beef in the U.S. *J. Sustain. Agric.* **33**, 189–209 (2009).
18. Keane, M. G. & Drennan, M. J. A comparison of Friesian, Aberdeen Angus×Friesian and Belgian Blue×Friesian steers finished at pasture or indoors. *Livest. Sci.* **115**, 268–278 (2008).
19. Butler, G. Manipulating dietary PUFA in animal feed: Implications for human health. *Proc. Nutr. Soc.* **73**, 87–95 (2014).
20. Maxin, G., Cornu, A., Andueza, D., Laverroux, S. & Graulet, B. Carotenoid, tocopherol and phenolic compound content and composition in cover crops used as forage. *J. Agric. Food Chem.* **68**, 6286–6296 (2020).
21. American Grassfed Association. *American Grassfed Association grassfed ruminant standards*. 1–19. https://www.americangrassfed.org/aga-grassfed-ruminant-standards/ (2022).
22. Booth, J., Howard, T., Miller-Auwerda, P. & Moore, J. Soybean hulls as an alternative feed for horses. **AS** 650, ASL R1931. https://lib.dr.iastate.edu/ans_air/vol650/iss1/90 (2004).
23. Kalač, P. The effects of feeding fresh forage and silage on some nutritional attributes of beef: An overview. *J. Agrobiol.* **28**, 1–13 (2011).
24. Clarke, E. D. et al. The relationship between dietary polyphenol intakes and urinary polyphenol concentrations in adults prescribed a high vegetable and fruit diet. *Nutrients* **12**, 3431 (2020).
25. Penczynski, K. J. et al. Relative validation of 24-h urinary hippuric acid excretion as a biomarker for dietary flavonoid intake from fruit and vegetables in healthy adolescents. *Eur. J. Nutr.* **56**, 757–766 (2017).
26. De Bellis, P., Maggiolino, A., Albano, C., De Palo, P. & Blando, F. Ensiling grape pomace with and without addition of a *Lactiplantibacillus plantarum* strain: Effect on polyphenols and microbiological characteristics, in vitro nutrient apparent digestibility, and gas emission. *Front. Vet. Sci.* **9**, 808293 (2022).
27. Kumar, N. & Goel, N. Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnol. Rep.* **24**, e00370 (2019).
28. Liu, S. X., Chen, D., Singh, M. & Xu, J. Extraction of proteins and pasting and antioxidant properties of soybean hulls. *J. Food Res.* **8**, 66–77 (2019).
29. Peiretti, P. et al. Phenolic composition and antioxidant activities of soybean (Glycine max (L.) Merr.) plant during growth cycle. *Agronomy* **9**, 153 (2019).
30. Kiesling, D. D. *Performance, carcass traits and fatty acid profiles of yearling beef cattle supplemented with self-fed byproducts on pasture* [master's thesis], (Iowa State University, 2013).
31. Kennedy, D. O. B vitamins and the brain: Mechanisms, dose and efficacy—A review. *Nutrients* **8**, 68 (2016).
32. Pighin, D. et al. A contribution of beef to human health: A review of the role of the animal production systems. *Sci. World J.* **2016**, 8681491 (2016).
33. 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 content. *J. Anim. Sci.* **87**, 2961–2970 (2009).
34. Logan, B. G., Hopkins, D. L., Schmidtke, L., Morris, S. & Fowler, S. M. Preliminary investigation into the use of Raman spectroscopy for the verification of Australian grass and grain fed beef. *Meat Sci.* **160**, 107970 (2020).
35. De la Fuente, J. et al. Fatty acid and vitamin E composition of intramuscular fat in cattle reared in different production systems. *Meat Sci.* **82**, 331–337 (2009).
36. Daley, C. A., Abbott, A., Doyle, P. S., Nader, G. A. & Larson, S. A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. *Nutr. J.* **9**, 1–12 (2010).
37. Alothman, M. et al. The "grass-fed" milk story: Understanding the impact of pasture feeding on the composition and quality of bovine milk. *Foods* **8**, 350 (2019).
38. Krusinski, L. et al. Fatty acid and micronutrient profile of *longissimus lumborum* from Red Angus and Red Angus x Akaushi cattle finished on grass or grain. *Foods* **11**, 3451 (2022).
39. Dewhurst, R. J., Shingfield, K. J., Lee, M. R. F. & Scollan, N. D. Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. *Anim. Feed Sci. Technol.* **131**, 168–206 (2006).
40. Kalač, P. & Samková, E. The effects of feeding various forages on fatty acid composition of bovine milk fat: A review. *Czech J. Anim. Sci.* **55**, 521–537 (2010).
41. Van Ranst, G., Fievez, V., Vandewalle, M., De Riek, J. & Van Bockstaele, E. Influence of herbage species, cultivar and cutting date on fatty acid composition of herbage and lipid metabolism during ensiling. *Grass Forage Sci.* **64**, 196–207 (2009).
42. Simopoulos, A. P. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed. Pharmacother.* **56**, 365–379 (2002).
43. Simopoulos, A. P. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: Nutritional implications for chronic diseases. *Biomed. Pharmacother.* **60**, 502–507 (2006).
44. Simopoulos, A. P. An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. *Nutrients* **8**, 128 (2016).
45. Simopoulos, A. P. Evolutionary aspects of diet: The omega-6/omega-3 ratio and the brain. *Mol. Neurobiol.* **44**, 203–215 (2011).
46. Harris, W. S. The omega-6:omega-3 ratio: A critical appraisal and possible successor. *Prostaglandins Leukot. Essent. Fat. Acids* **132**, 34–40 (2018).
47. Prache, S. Diet authentication in sheep from the composition of animal tissues and products. *Rev. Bras. de. Zootec.* **38**, 362–370 (2009).
48. Carrillo, J. A. et al. Integrated metabolomic and transcriptome analyses reveal finishing forage affects metabolic pathways related to beef quality and animal welfare. *Sci. Rep.* **6**, 25948 (2016).
49. Prache, S., Martin, B. & Coppa, M. Review: Authentication of grass-fed meat and dairy products from cattle and sheep. *Animal* **14**, 854–863 (2020).
50. Monahan, F. J., Schmidt, O. & Moloney, A. P. Meat provenance: Authentication of geographical origin and dietary background of meat. *Meat Sci.* **144**, 2–14 (2018).
51. Bleu-Blanc-Coeur. Production et transformation de bovin viande. Cahier des ressources. https://bleu-blanc-coeur.org/a-propos/nos-cahiers-des-ressources/ (2020).
52. Manso, T. et al. Influence of dietary grape pomace combined with linseed oil on fatty acid profile and milk composition. *J. Dairy Sci.* **99**, 1111–1120 (2016).
53. Verduna, T., Blanc, S., Merlino, V. M., Cornale, P. & Battaglini, L. M. Sustainability of four dairy farming scenarios in an Alpine environment: The case study of Toma di Lanzo cheese. *Front. Vet. Sci.* **7**, 569167 (2020).
54. Campbell, K. L., Moore, J. B. & Bartholomew, J. B. The importance of publishing null results: Editorial guidelines to contribute to the reduction of publication bias in translational exercise research. *Trans. J. Am. Coll. Sports Med.* **5**, 1 (2020).
55. Sampels, S. *Oxidation and antioxidants in fish and meat from farm to fork*, 115–144 (IntechOpen, 2013).
56. Huang, X. & Ahn, D. U. Lipid oxidation and its implications to meat quality and human health. *Food Sci. Biotechnol.* **28**, 1275–1285 (2019).
57. Neri, L. et al. Antioxidant activity in frozen plant foods: Effect of cryoprotectants, freezing process and frozen storage. *Foods* **9**, 1886 (2020).
58. Maciel, I. C. F. et al. Influence of beef genotypes on animal performance, carcass traits, meat quality, and sensory characteristics in grazing or feedlot-finished steers. *Transl. Anim. Sci.* **5**, txab214 (2021).
59. Mertens, D. R. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. *J. AOAC Int.* **85**, 1217–1240 (2002).
60. AOAC. *Official methods of analysis* (The Association of Official Analytical Chemists, 2000).
61. Hach, C. C., Bowden, B. K., Kopelove, A. B. & Brayton, S. V. More powerful peroxide Kjeldahl digestion method. *J. Assoc. Anal. Chem.* **70**, 783–787 (1987).
62. Lichtenthaler, H. K. & Wellburn, A. R. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* **11**, 591–592 (1983).
63. Nimalaratne, C., Lopes-Lutz, D., Schieber, A. & Wu, J. Free aromatic amino acids in egg yolk show antioxidant properties. *Food Chem.* **129**, 155–161 (2011).
64. Singleton, V. L. & Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **16**, 144–158, (1965).
65. Sergin, S. et al. Fatty acid and antioxidant composition of conventional compared to pastured eggs: Characterization of conjugated linoleic acid and branched chain fatty acid isomers in eggs. *Food Sci. Technol.* **1**, 260–267 (2021).
66. Jenkins, T. C. Technical note: Common analytical errors yielding inaccurate results during analysis of fatty acids in feed and digesta samples. *J. Dairy Sci.* **93**, 1170–1174 (2010).
67. Kramer, J. K., Hernandez, M., Cruz-Hernandez, C., Kraft, J. & Dugan, M. E. Combining results of two GC separations partly achieves determination of all cis and trans 16:1, 18:1, 18:2 and 18:3 except CLA isomers of milk fat as demonstrated using Ag-ion SPE fractionation. *Lipids* **43**, 259–273 (2008).
68. Rettenmaier, R. & Schüep, W. Determination of vitamins A and E in liver tissue. *Int. J. Vitam. Nutr. Res.* **62**, 312–317 (1992).
69. Wahlen, R., Evans, L., Turner, J. & Hearn, R. The use of collision/reaction cell ICP-MS for the determination of elements in blood and serum samples. *Spectroscopy* **20**, 84–89, (2005).
70. van Vliet, S. et al. A metabolomics comparison of plant-based meat and grass-fed meat indicates large nutritional differences despite comparable Nutrition Facts panels. *Sci. Rep.* **11**, 13828 (2021).
71. Bifarin, O. O. Interpretable machine learning with tree-based shapley additive explanations: Application to metabolomics datasets for binary classification. *PLoS ONE* **18**, e0284315 (2023).

Acknowledgements

We thank the Kellogg Biological Station (KBS) at Michigan State University for allowing us to use their land and managing our animals for the length of the trial. We also express our gratitude to the Michigan State University College of Agriculture and Natural Resources Statistical Consulting Center (CANR SCC) for their help with the statistical analysis. While at Chapman University, L.K. was supported by a fellowship from the Grand Challenges Initiative. Finally, we also thank the Greenacres Foundation for their financial support that allowed us to complete this work. The funder had no influence on the design of the study and collection, analysis, and interpretation of data, or the writing of this manuscript.

Author contributions

L.K.: Conceptualization, Methodology, Formal analysis and data curation, Statistical analysis, Writing–original draft preparation, Writing–review and editing. I.C.F.M.: Conceptualization, Formal analysis and data curation, Writing–review and editing, Supervision. S.v.V.: Conceptualization, Methodology, Formal analysis and data curation, Statistical analysis, Writing–review and editing, Supervision. M.A.: Methodology, Formal analysis and data curation, Writing–review and editing. J.A.: Formal analysis and data curation, Statistical analysis, Writing–original draft preparation, Writing–review and editing. G.L.: Formal analysis and data curation, Statistical analysis, Writing–review and editing. C.A.B.: Writing–review and editing, Funding acquisition. J.E.R.: Conceptualization, Methodology, Writing–review and editing, Supervision, Funding acquisition. J.I.F.: Conceptualization, Methodology, Formal analysis and data curation, Statistical analysis, Writing–original draft preparation, Writing–review and editing, Supervision, Funding acquisition. All authors read and approved the final manuscript.

Competing interests

L.K. reports grants from NCR-SARE (GNC21-328) and the Greenacres Foundation to investigate the effects of different feedstuffs on the nutrient density of beef. J.E.R. and J.I.F. report grants from NCR-SARE and the Greenacres Foundation for the enhancement of the healthfulness and demand of Upper-Midwestern, locally produced beef. L.K., J.E.R., and J.I.F. have not accepted personal honoraria from any organization to prevent the undue influence in the eye of the public. S.v.V. reports grant support from USDA (2020-38640-31521; 2021-67034-35118; 2022-58-3064-2-007), the Turner Institute of Ecoagriculture, Perdue Foods LLC, the Bionutrient Institute, and the Greenacres Foundation. S.v.V. also reports having received speaking fees and travel reimbursements for talks related linking food production systems to human health. C.A.B. is part of the Greenacres Foundation. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be considered as potential conflicts of interest. The funder (Greenacres Foundation) had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41538-024-00315-5.

Correspondence and requests for materials should be addressed to Jenifer I. Fenton.