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Range-Based Finishing Enhances Omega-3 Fatty Acids and Forage- Derived Metabolites in Bison Meat Across a Seven Treatment Comparison

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Abstract

Background

Finishing practices influence the biochemical composition of bison (*Bison bison*) meat, with potential implications for animal health and human nutrition. This study evaluated seven representative finishing systems spanning a range of extensive and intensive production systems, with additional contrasts in forage diversity, corn supplementation, and stocking density to determine their effects on the fatty acid and phytonutrient profiles of bison striploins (*M. longissimus lumborum*). Treatments included range-finished bison grazing either diverse native pastures (~ 200 plant species) or lower-diversity pastures (~ 90 plant species), with or without corn supplementation, as well as pen-finished bison managed under varying feed formulations and stocking densities, including a conventional total mixed ration group. Fatty acids were analyzed by gas chromatography–flame ionization detection (GC-FID), and targeted metabolomic profiling of phytonutrient-derived metabolites was performed using liquid chromatography–tandem mass spectrometry (LC–MS/MS).

Results

Range-finished bison exhibited significantly higher concentrations of alpha-linolenic acid (ALA) (C18:3n-3; $P < 0.0001$), DPA (C22:5n-3; $P < 0.01$), and DHA (C22:6n-3; $P < 0.0001$), and lower n-6:n-3 ratios (2.8–3.6) than pen-finished bison (5.0–7.3; $P < 0.0001$). Metabolomic profiling revealed enrichment of hippuric acid, catechol, and shikimic acid (candidate biomarkers associated with forage intake and polyphenolic metabolism) in range-finished systems, whereas pen-finished bison exhibited higher concentrations of 3-(4-hydroxyphenyl)lactic acid, a potential grain-associated metabolite (all, $P < 0.05$).

Conclusions

Range-finished systems enhanced omega-3 fatty acid and phytonutrient-related metabolite profiles, whereas the conventional TMR feedlot treatment yielded the lowest levels and the free-choice corn-and-hay pen system yielded an intermediate profile. Overall, the clearest nutritional contrast was between range-finished and pen-finished systems, whereas differences attributable to forage diversity or stocking density were comparatively modest. These findings indicate that finishing system strongly influences the biochemical composition of bison meat, although comparisons involving the conventional TMR feedlot treatment should be interpreted cautiously because that group originated from a separate breeding herd.

BACKGROUND

The nutrient composition of red meat is increasingly recognized as a function of animal genetics and management systems [1]. Among these, finishing strategies, including diet type, forage diversity, and stocking density, play a potential role in determining the biochemical quality of meat by affecting lipid

metabolism, ruminal biohydrogenation [2], and exposure to plant primary and secondary metabolites [3]. In ruminant species, forage-based diets typically enhance the concentrations of eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA), conjugated linoleic acid (CLA), and phytochemicals, while decreasing n-6:n-3 ratios relative to grain-based feeding systems [4, 5]. These compositional differences have potential implications for human health, as a higher dietary intake of n-3 PUFAs and lower n-6:n-3 ratios are associated with improved cardiometabolic and inflammatory profiles [6], and circulating omega-3 fatty acids in consumers can be increased through the consumption of ruminant-based meats[7].

The North American bison industry encompasses a continuum of production systems, ranging from extensive native rangeland grazing and finishing (i.e., grass-finishing) to confinement finishing on total mixed rations (TMR; i.e., grain-finishing) [8, 9]. This diversity reflects both ecological adaptation and economic strategy but also results in considerable variation in ruminant meat nutritional traits [10]. Previous studies on beef and lamb have found that the finishing environment can markedly alter the tissue fatty acid composition and metabolite signatures [11, 12]. Recent work on bison has similarly found that pasture finishing enhances long-chain n-3 fatty acids and phytonutrient antioxidants, whereas confinement systems promote higher oleic and n-6 fatty acids, mirroring the findings in beef [13]. However, there is limited integrated evidence linking diverse finishing environments (including variations in forage composition, feed supplementation, and stocking density) to fatty acid and phytochemical composition of bison meat.

Beyond lipids, emerging metabolomic evidence finds that meat from range-finished animals contains distinct signatures of plant-derived and microbial metabolites, such as hippuric acid, cinnamic acid, and p-cresol metabolites, which reflect the intake and transformation of phenolics in the rumen and host tissues [14]. These metabolites may serve as biomarkers of production authenticity and have been suggested to be functionally relevant to human nutrition through antioxidant or anti-inflammatory actions, although their direct impact on human health via meat consumption requires further investigation [15]. Nonetheless, various plant secondary metabolites (e.g., flavonoids, non-flavonoid polyphenols, and simple phenols) have been found to benefit animal health and welfare due to their potential anti-inflammatory [16], anti-parasitic [17], and antioxidant effects [18].

The current study builds upon the recent work of O'Sullivan et al. (2025), who comprehensively characterized the carcass and sensory attributes of bison heifers finished under the different management systems studied here [8]. While that study focused on proximate composition and palatability, the present work extends those findings by examining the underlying biochemical and nutritional traits of meat derived from the same finishing treatments. Therefore, the objective of this study was to evaluate the impact of seven representative finishing systems, ranging from range-finishing to high-density confinement feeding, on the fatty acid composition and metabolomic profile of bison striploins. We hypothesized that bison finished under range conditions would exhibit lower n-6:n-3 ratios and higher concentrations of forage-associated phytochemical metabolites than those finished under pen-based TMR systems, and that corn supplementation on pasture would yield an intermediate profile.

By linking the production environment to quantifiable biochemical outcomes, this study aimed to provide mechanistic insights into how ecological management and nutrient density are connected within bison production systems.

MATERIALS AND METHODS

Bison (*Bison bison*) heifers born in spring 2021 were studied in seven finishing systems designed to represent a range of management practices used in the North American bison industry. These systems varied primarily in diet composition and finishing environment, with additional contrasts in forage diversity and stocking density, to evaluate their effects on the fatty acid and phytonutrient profiles of bison meat (Fig. 1). Treatments 1–4 represented confinement-finishing environments: Pen-FREE-LOW (Treatment 1; n = 45) with free-choice access to alfalfa hay, meadow hay, and corn at low stocking density (55 m² per animal); Pen-TMR-LOW (Treatment 2; n = 43), a bison total mixed ration (TMR) prepared from corn and oats mixed with ground alfalfa hay, ground grass hay, and added water, offered at low stocking density; Pen-TMR-HIGH (Treatment 3; n = 44), the same TMR offered at high stocking density (27 m² per animal); and Pen-TMR-TRAD (Treatment 4; n = 45), a commercial high-density feedlot system typical of conventional bison production. Feed ingredients were analyzed by wet chemistry and adjusted during the trial in treatments 1–3 as needed to meet finishing requirements (Table 1).

Table 1
Standard Feed Analysis of Rations Used in the Bison Heifer Finishing Trial (100% Dry Matter Basis)

Treatment	Feed Type / Description	DM (%)	CP (%)	NDF (%)	ADF (%)	TDN (%)	NEm (Mcal kg^{-1})	RFV
1a	Pen-FREE-LOW – Whole corn	85.6	8.6	8.3	2.7	86.6	2.14	—
1b	Pen-FREE-LOW – Grass hay	90.1	10.4	67.1	42.0	55.2	1.15	78
1c	Pen-FREE-LOW – Alfalfa hay	82.3	20.2	53.5	42.2	53.4	1.08	97
2 & 3	Pen-TMR-LOW / Pen-TMR-HIGH (bison total mixed ration)	73.0	12.6	25.1	16.1	76.9	1.85	—
4	Pen-TMR-TRAD (commercial high-density feedlot ration)*	—	—	—	—	—	—	—
5	Range-DIV (diverse native rangeland forage)	84.1	4.5	70.0	44.1	53.4	1.08	72
6	Range-LIM (low-diversity rangeland forage)	70.5	4.4	66.3	44.6	52.5	1.06	76
7	Range-LIM-CORN (low-diversity meadow forage)	65.4	6.8	65.6	43.2	54.3	1.12	78

Feed composition values are presented on a 100% dry-matter basis. Samples were collected on 22 November 2023 and analyzed on 2 December 2023. Abbreviations include: DM (dry matter), CP (crude protein), NDF (neutral detergent fiber), ADF (acid detergent fiber), TDN (total digestible nutrients), NEm (net energy for maintenance), and RFV (relative feed value). RFV values apply exclusively to forage components (Treatments 1b, 1c, 5, 6, and 7) and were not determined for total mixed rations (TMR) or corn samples. A representative feed sample was collected for Treatment 4 (Pen-TMR-TRAD), but proximate composition values were not available at the time of analysis.

Treatments 5–7 represented extensive pasture-finishing environments: Range-DIV (Treatment 5; n = 44) grazing native upland rangeland characterized by high botanical diversity (an estimated 200 + plant species) within a mixed-grass prairie containing grasses, forbs, and sedges; Range-LIM (Treatment 6; n = 44) grazing lowland meadow pasture with lower forage diversity (90 + plant species) dominated by sedge and grass species; and Range-LIM-CORN (Treatment 7; n = 43), a similar low-diversity pasture with free-choice access to whole-kernel corn. Animals in these treatments were managed at very low stocking densities to optimize forage utilization and prevent overgrazing. Water was provided ad libitum.

Botanical diversity of the rangeland pastures (high vs. low) was visually assessed and represents the observations and experience of the staff at McGinley Ranch regarding the abundance of plant species.

All animals in Treatments 1–3 and 5–7 originated from the same breeding herd and were managed under low-stress handling protocols throughout the trial period at McGinley Ranch. Treatment 4 animals originated from a separate breeding herd and were finished at a commercial feedlot in South Dakota, USA, using a grain-based TMR. All pens (treatments 1–3) at McGinley Ranch were open-air, and feed and water availability were monitored daily to maintain ad libitum intake.

Prior to assignment, the bison were vaccinated in February 2022 (ViraShield 6 + Somnus, Mycoplasma, Alpha7/MB-1, Cydectin injection) and October 2022 (Safe-Guard drench, Nuplura, Mycoplasma). The heifer calves remained with mother cows until fall 2022 and were then separated from the breeding herd and maintained separately on pasture until treatment allocation. The animals were visually assessed daily for health and behavior across all systems throughout the finishing period. Finishing commenced in May 2023 and continued for approximately seven months until harvest in December 2023–January 2024.

At the conclusion of the finishing period, the bison were transported to a USDA-inspected commercial slaughter facility. The animals were stunned and dispatched by rapid exsanguination. Transportation distances ranged from approximately 402 km for McGinley Ranch animals (Treatments 1–3 and 5–7) to 175 km for animals finished under the traditional feedlot system (Treatment 4), which were all slaughtered in the same facility. Live weight and hot carcass weight (HCW) were recorded before and after slaughter, respectively. After a 24-h chilling period, carcasses were ribbed between the 12th and 13th ribs, and ribeye area, backfat thickness, marbling score, and kidney fat percentage were determined by trained personnel according to the USDA beef grading guidelines reported by O'Sullivan et al. (2025). Heifers were harvested when visually appraised to be at the targeted degree of finish. Consequently, range-finished animals required an additional month on pasture before reaching the targeted finish compared to pen-finished animals.

The *M. longissimus lumborum* (striploin) was removed from the left side of a subsample of carcasses from each treatment (n = 30 carcasses closest to the average HCW per treatment), vacuum-packaged, and transported under refrigeration to the South Dakota State University Meat Laboratory for further fabrication and analysis. Upon arrival, the striploins were trimmed of external fat and fabricated into 2.54-cm steaks. One steak from each of 16 carcasses (n = 16) closest to the average HCW per treatment was aged for 14 d postmortem at 4°C, vacuum-sealed, frozen (− 20°C), and sent to the Center for Human Nutrition at Utah State University for fatty acid and phytonutrient analysis. All samples were stored and handled under identical conditions to minimize the variability associated with postmortem storage or temperature fluctuations. For compositional assays, the steaks were thawed at 4°C for 24 h, homogenized using a stainless-steel grinder, and allocated for fatty acid methyl ester (FAME) preparation and targeted metabolomic profiling. Tissue aliquots were flash-frozen in liquid nitrogen and stored at − 80°C until extraction.

Representative feed and forage samples were collected during the finishing period for fatty acid and phytonutrient analyses. For Pen-FREE-LOW, separate samples of alfalfa hay, meadow hay, and whole corn were collected. Because these ingredients were offered free-choice, the Pen-FREE-LOW values shown in Table 2 were calculated as a dry-matter-weighted treatment-level composite of the offered ingredients rather than as a single consumed ration profile; oats recorded in the feed log were included within the grain fraction because a separate oats fatty acid sample was not collected for analysis. Pen-TMR-LOW and Pen-TMR-HIGH received the same bison TMR formulation (corn and oats mixed with ground alfalfa hay, ground grass hay, and added water), and a representative TMR sample was therefore used for both treatments. Representative samples were also collected from the traditional feedlot TMR and from the forages associated with the Range-DIV and Range-LIM treatments. For Range-LIM-CORN, Table 2 values were calculated as a treatment-level composite of low-diversity meadow forage plus the sampled corn supplement. Feed and forage samples were freeze-dried, ground to a uniform powder, and stored at − 80°C until fatty acid and targeted metabolomic analyses.

Table 2
Feed Fatty Acid Composition (data shown as % fatty acids)

Fatty acid	Pen-FREE-LOW[1]	Pen-TMR-LOW[1]	Pen-TMR-HIGH[1]	Pen-TMR-TRAD[1]	Range-DIV[1]	Range-LIM[1]	Range-LIM-CORN[1]
Myristic	0.25	0.19	0.19	0.14	2.23	1.72	0.46
Palmitic	16.44	16.60	16.60	14.49	21.87	23.02	16.36
Palmitoleic acid (trans)	0.44	0.26	0.26	0.12	0.96	1.00	0.39
Palmitoleic acid (cis)	0.13	0.16	0.16	0.15	0.19	0.27	0.15
Stearic	2.34	2.17	2.17	3.21	4.39	2.98	2.04
Elaidic acid	0.06	0.07	0.07	0.09	0.13	0.09	0.07
Oleic acid	22.47	22.84	22.84	23.96	12.46	6.64	21.89
Linoleic acid	49.70	51.39	51.39	52.27	29.98	22.46	44.04
Arachidic acid	0.61	0.56	0.56	0.48	3.01	1.64	0.77
Gamma linolenic acid	0.08	0.06	0.06	0.04	0.21	0.32	0.10
Alpha-linolenic acid	6.84	5.04	5.04	4.53	22.13	37.95	13.10
Behenic acid	0.65	0.66	0.66	0.52	2.44	1.92	0.63

[1]Treatments: Pen-FREE-LOW = free-choice alfalfa/hay/corn, low stocking density; Pen-TMR-LOW = bison total mixed ration (TMR), low density; Pen-TMR-HIGH = bison TMR, high density; Pen-TMR-TRAD = traditional TMR, high density (feedlot); Range-DIV = diverse native range; Range-LIM = low-diversity range; Range-LIM-CORN = low-diversity range + corn. Pen-FREE-LOW is shown as a single treatment column for manuscript presentation; values represent a dry-matter-weighted treatment-level composite of the offered alfalfa hay, grass hay, and grain fraction rather than a single consumed ration profile. Range-LIM-CORN values represent a treatment-level composite of low-diversity meadow forage plus the corn supplement. Pen-TMR-LOW and Pen-TMR-HIGH received the same TMR formulation, so values are identical because stocking density, not diet composition, was the experimental variable.

Fatty Acid Analysis

The fatty acid composition of *M. longissimus lumborum* and feed samples was determined by gas chromatography following lipid extraction and methylation into fatty acid methyl esters (FAMEs) according to the one-step method described by O'Fallon et al. [19]. Approximately 250 mg of homogenized muscle or ground feed/forage tissue was weighed into 16 × 125 mm screw-cap tubes, and 350 µL of 10 N KOH in methanol and 3,150 µL of methanol were added, along with an internal standard

(C13:0; Nu-Chek Prep, Elysian, MN, USA). The samples were vortexed and incubated in a water bath at 55°C for 1.5 h with intermittent agitation every 20 min. Following saponification, 290 µL of 24 N H_2SO_4 in methanol was added to induce methylation, and the samples were reincubated at 55°C for an additional 1.5 h. After cooling to room temperature, 2 mL of hexane containing 0.01% butylated hydroxytoluene (BHT) and 1 mL of deionized water were added to the mixture. The tubes were vortexed for 30 s and centrifuged at 1,000 × g for 5 min to separate the organic phase. The upper hexane layer containing the FAMEs was transferred to autosampler vials for gas chromatographic analysis. FAMEs were quantified using a Shimadzu GC-2010 Plus Gas Chromatograph (Shimadzu Scientific Instruments, Columbia, MD, USA) equipped with a flame ionization detector and a 100 m × 0.25 mm i.d., 0.25 µm film thickness SP-2560 capillary column (Supelco, Bellefonte, PA, USA). The injector and detector temperatures were maintained at 250°C and 260°C, respectively. The oven temperature program was as follows: initial 140°C (hold 5 min), ramped at 5°C min^{-1} to 240°C, and held for 20 min. Helium served as the carrier gas at a constant flow rate of 1 mL min^{-1}. Extracted FAMEs were analyzed by gas chromatography with flame ionization detection, and concentrations were expressed as percentages of total identified fatty acids. Individual fatty acids were identified by comparing retention times with a 37-component FAME standard mix (Supelco 47885-U) and expressed as a percentage of the total identified fatty acids. Fatty acids were grouped by saturation class as saturated (SFA), monounsaturated (MUFA), or polyunsaturated (PUFA), and by family (n-3 and n-6 fatty acids). The n-6:n-3 ratio was calculated as the sum of n-6 divided by the sum of n-3 fatty acids. All chromatograms were processed using the Shimadzu LabSolutions software, and the peaks were manually inspected for integration accuracy. Fatty acid data were reported on a normalized basis (% of total FAMEs) for all the subsequent statistical analyses.

Phytonutrient Analysis

Targeted metabolomic profiling quantified 43 phytonutrient metabolites in *M. longissimus lumborum* and 144 metabolites in feed samples. One hundred milligrams of fresh meat tissue and 30 mg of freeze-dried plant tissue were weighed into 2 mL tubes. A 5 mm stainless steel bead and 1,250 µL of pre-cooled (− 20°C) internal standard-spiked extraction solvent containing MTBE:MeOH (2:1, v/v) were added to each sample. Isotopically labeled internal standards included 4-hydroxybenzoic acid-13C6, apigenin-d_5, benzoic acid-d_5, genistein-d_5, resveratrol-$^{13}C_6$, phenol-$^{13}C_6$, and trans-cinnamic acid-d_7. Samples were lysed using a bead-beater set to 25 oscillations per second for 2 min. Samples were then mixed with 750 µL of water, vortexed, and centrifuged at 16,000 rcf for 10 min at 4°C to induce phase separation. The MTBE layer was removed, and the lower phase was collected in a new set of tubes. Thereafter, 500 µL of methanol were added, and samples were stored at − 80°C overnight to facilitate protein precipitation. The next day, samples were centrifuged at 16,000 rcf for 10 min at 4°C and passed through a 0.22 µm PTFE filter. Finally, samples were stored at − 20°C for 4 h, followed by a final centrifugation at 16,000 rcf for 10 min at 4°C, after which they were transferred to HPLC vials and stored at − 80°C until injection. For plant samples, the remaining pellet was subsequently washed with 1,000 µL of methanol, and 1,250 µL of internal standard-spiked 2 M NaOH (same internal standards as the MTBE:MeOH described prior) was added for alkaline hydrolysis for 16 h in a water bath at 30°C. The hydrolyzed plant extract containing

bound phenolics was then acidified to pH 3 with concentrated formic acid and sonicated for 10 min at room temperature. Samples were then centrifuged at 16,000 rcf for 10 min at 4°C, filtered through a 0.22 µm membrane, and subjected to solid-phase extraction (SPE) using a Strata-X-Pro 96-well plate. During SPE, samples were washed with 1,250 µL of water in 0.1% formic acid and eluted with 1,250 µL of methanol in 0.1% formic acid. Compounds were separated using a Waters ACQUITY UPLC 1.7 µm HSS T3 column (100 × 2.1 mm) with a column temperature maintained at 30°C. The mobile phases consisted of (A) water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid. The gradient elution program was as follows: 5% B for 2.1 minutes at 0.2 mL/min, ramping to 95% B over 14 minutes at a flow rate of 0.46 mL/min, followed by re-equilibration to 5% B for 4 minutes at 0.175 mL/min to maintain stable system pressure. All analytes were initially prepared as stock solutions ranging from 1 to 10 mM in appropriate solvents based on solubility. Multiple reaction monitoring (MRM) methods were developed by direct infusion of each standard compound (1–5 µM) using a 1 mL syringe at a flow rate of 5–20 µL/min to attain Q1 and Q3 transitions, CE, CXP, and Q0D, and were categorized based on ionization efficiency in positive and negative mode. The ion source conditions for both positive and negative assays were optimized separately using 15 representative phytonutrient standards for each mode. For negative mode, the optimized parameters were source temperature 550°C, spray voltage − 5500 V, curtain gas 40 psi, CAD 12, and ion source gases 1 and 2 at 70 psi. For positive mode, the spray voltage was set to + 5000 V. The MRM cycle time was set to 1000 ms, with dwell times ranging from 3–40 ms depending on the number of transitions monitored.

Quality control and data analysis

Quality control (QC) measures and calibration procedures were implemented in this study as per best practice in metabolomics studies. Specifically, after every 15 sample injections, a double blank (100% methanol) and a blank spiked only with isotopically labeled internal standards were injected as QC samples. All samples were processed in analytical batches of 24. Samples were randomized prior to instrumental analysis to minimize the batch effects and analytical bias. Data acquisition and processing were performed using the SCIEX OS software version 3.0 (SCIEX, Framingham, MA, USA). Peak integration was performed using the MQ4 algorithm with Gaussian smoothing set to a factor of 3. Compound identification was confirmed based on the presence of both quantifier and qualifier ion transitions and ion ratio consistency. A signal-to-noise (S/N) threshold of 10 was applied, and a noise percentage filter of 80% was used to ensure robust peak detection. Quantitative calibration curves were validated with a minimum regression coefficient (R^2) of 0.90, in accordance with the standard metabolomics quality assurance guidelines.

Statistical Analysis

Fatty acid data were analyzed using GraphPad Prism version 10.1.1 (GraphPad Software, San Diego, CA, USA), whereas phytonutrient data were analyzed using MetaboAnalyst 6.0. Differences among the seven finishing systems were determined using one-way analysis of variance (ANOVA), with the finishing

system as a fixed effect. When ANOVA indicated significance ($P < 0.05$), Tukey's multiple comparisons test was applied to identify pairwise differences among treatments while hierarchical clustering heatmaps were generated using MetaboAnalyst 6.0. Fatty acid data were expressed as the percentage of total identified fatty acids and n-6:n-3 ratios were calculated as the sum of n-6 divided by the sum of n-3 fatty acids. Individual and grouped fatty acids (SFA, MUFA, and PUFA) were analyzed separately. Metabolite concentrations were reported as µg/100 g wet weight for meat. Individual plant metabolites are reported in Table S2 as µg/100 g dry weight; summed total phenolic concentrations derived from those values are discussed in the text as mg/100 g dry weight. All results are presented as means ± SEM, and significance was declared at $P < 0.05$, with trends discussed at $0.05 \leq P \leq 0.10$. Pearson correlation analyses were performed in Python using the SciPy library to assess pairwise associations among meat metabolites at the individual-sample level ($n = 108$ after exclusion of 4 samples with incomplete metabolite data). For treatment-mean forage-to-meat biomarker associations ($n = 7$; Fig. 6), Spearman rank correlation analyses were used. Correlation heatmaps and scatter plots, including multi-panel layouts, were generated in Python using the Matplotlib library.

RESULTS

Fatty Acid Composition

The fatty acid compositions of the feeds used across different finishing systems showed notable variation (Table 2). Range-associated feed profiles (Range-DIV, Range-LIM, and the corn-supplemented Range-LIM-CORN composite) contained higher concentrations of alpha-linolenic acid (ALA; C18:3n-3) than pen-based TMR rations, with values of 22.13%, 37.95%, and 13.10%, respectively, compared with values ranging from 4.53% to 5.04% in Pen-TMR-LOW, Pen-TMR-HIGH, and Pen-TMR-TRAD. Pen-FREE-LOW provided free-choice access to both hay and corn and therefore should be interpreted as a mixed-diet pen system rather than as a hay-based comparison. Accordingly, the PEN-FREE-LOW values in Table 2 are best viewed as a dry-matter-weighted treatment-level composite of the offered ingredients rather than as a single consumed ration profile. All pen-based TMR diets exhibited elevated linoleic acid (C18:2n-6) levels, exceeding 50%.

Range-finished bison (Range-DIV, Range-LIM, and Range-LIM-CORN) displayed higher levels of n-3 fatty acids than the pen-finished groups (Table 3; Table S1; Fig. 2). Specifically, concentrations of alpha-linolenic acid (ALA; C18:3n-3), docosapentaenoic acid (DPA, C22:5n-3), and docosahexaenoic acid (DHA, C22:6n-3) were significantly higher in all range-finished bison (ALA and DHA, $P < 0.0001$; DPA, $P < 0.01$), whereas eicosapentaenoic acid (EPA, C20:5n-3) was slightly higher overall in pen-finished animals ($P < 0.0001$; Table 3; Fig. 2D). Alpha-linolenic acid (C18:3n-3) concentrations ranged from 2.58 ± 0.10% in Range-DIV to 2.87 ± 0.11% in Range-LIM, compared to 0.37 ± 0.04% to 0.71 ± 0.03% in pen-finished animals (Table 3). DPA (C22:5n-3) levels were 1.97 ± 0.13% in Range-DIV and 1.97 ± 0.09% in Range-LIM, versus 0.53 ± 0.03% to 0.91 ± 0.06% in pen-finished groups. The total n-3 fatty acid concentrations (∑ n-3) were 5.22 ± 0.27% and 5.49 ± 0.21% in Range-DIV and Range-LIM, respectively, compared to 1.28 ± 0.04% to 2.13 ± 0.13% in pen-finished systems. Additionally, the n-6:n-3 ratios were substantially lower in the

range systems (2.8–3.6) than in the pen-fed animals (5.0–7.3). Specifically, Range-DIV and Range-LIM exhibited ratios of 2.88 ± 0.06 and 2.83 ± 0.05, respectively, whereas pen-finished animals showed ratios ranging from 5.03 ± 0.11 (Pen-TMR-HIGH) to 7.25 ± 0.30 (Pen-TMR-TRAD) (Table 3). The inclusion of corn in the Range-LIM-CORN group led to reduced n-3 levels compared with the diverse native range (Range-DIV), with total n-3 concentrations of 2.34 ± 0.16% and an n-6:n-3 ratio of 3.61 ± 0.07 in Range-LIM-CORN versus 5.22 ± 0.27% and 2.88 ± 0.06 in Range-DIV, indicating that adding grain on pastures modulates fatty acid profiles.

Table 3
Effect of finishing systems on meat fatty acid composition.

Fatty acid	Pen-FREE-LOW[1]	Pen-TMR-LOW[1]	Pen-TMR-HIGH[1]	Pen-TMR-TRAD[1]	Range-DIV[1]	Range-LIM[1]	Range-LIM-CORN[1]	P Value[2,3]
C18:2 (LA)	5.84 ± 0.31^{b}	6.25 ± 0.48^{b}	7.24 ± 0.49^{b}	6.59 ± 0.33^{b}	9.19 ± 0.56^{a}	9.69 ± 0.43^{a}	5.49 ± 0.41^{b}	< 0.0001
C18:3 (ALA)	0.56 ± 0.03^{de}	0.58 ± 0.03^{de}	0.71 ± 0.03^{ce}	0.37 ± 0.04^{d}	2.58 ± 0.10^{b}	2.87 ± 0.11^{a}	0.88 ± 0.06^{c}	< 0.0001
C20:4 (AA)	0.42 ± 0.03^{bd}	0.44 ± 0.04^{bd}	0.51 ± 0.04^{bd}	0.25 ± 0.02^{cd}	1.48 ± 0.11^{a}	1.54 ± 0.08^{a}	0.60 ± 0.05^{b}	< 0.0001
C20:5 (EPA)	0.22 ± 0.01^{acd}	0.23 ± 0.02^{ac}	0.26 ± 0.02^{a}	0.26 ± 0.02^{a}	0.21 ± 0.01^{acd}	0.19 ± 0.01^{bcd}	0.17 ± 0.01^{bd}	< 0.0001
C22:5 (DPA)	0.74 ± 0.06^{bd}	0.77 ± 0.05^{bd}	0.91 ± 0.06^{b}	0.53 ± 0.03^{cd}	1.97 ± 0.13^{a}	1.97 ± 0.09^{a}	0.99 ± 0.08^{b}	< 0.01
C22:6 (DHA)	0.18 ± 0.02^{cde}	0.20 ± 0.03^{bde}	0.24 ± 0.02^{be}	0.13 ± 0.01^{cd}	0.45 ± 0.04^{a}	0.46 ± 0.03^{a}	0.30 ± 0.03^{b}	< 0.0001
∑ n-6	8.74 ± 0.51^{b}	9.31 ± 0.74^{b}	10.80 ± 0.77^{b}	9.29 ± 0.47^{b}	15.20 ± 1.01^{a}	15.62 ± 0.71^{a}	8.53 ± 0.65^{b}	< 0.0001
∑ n-3	1.69 ± 0.11^{bd}	1.79 ± 0.12^{bd}	2.13 ± 0.13^{b}	1.28 ± 0.04^{cd}	5.22 ± 0.27^{a}	5.49 ± 0.21^{a}	2.34 ± 0.16^{b}	< 0.0001
n-6:n-3 ratio	5.20 ± 0.09^{b}	5.15 ± 0.12^{b}	5.03 ± 0.11^{b}	7.25 ± 0.30^{a}	2.88 ± 0.06^{d}	2.83 ± 0.05^{d}	3.61 ± 0.07^{c}	< 0.0001

[1]*Treatments: Range-DIV = diverse native range; Range-LIM = low-diversity range; Range-LIM-CORN = low-diversity range + corn; Pen-FREE-LOW = free-choice alfalfa hay, meadow hay, and corn at low stocking density; Pen-TMR-LOW = bison total mixed ration (TMR) at low stocking density; Pen-TMR-HIGH = the same TMR at high stocking density; Pen-TMR-TRAD = traditional TMR at high stocking density (feedlot).*

[2]*P values indicate the overall treatment effect across the seven finishing systems; pairwise comparisons were assessed using Tukey's test when the overall effect was significant.* Within a row, treatment means that do not share a common superscript letter differ significantly (P < 0.05).

[3]*Values are expressed as mean ± SEM (% of total fatty acids); n = 16 animals per treatment.*

Linoleic acid (C18:2n-6) concentrations varied considerably across the finishing systems. Pen-finished animals had concentrations ranging from 5.84 ± 0.31% (Pen-FREE-LOW) to 7.24 ± 0.49% (Pen-TMR-HIGH). Among the range-finished bison, Range-DIV and Range-LIM exhibited higher linoleic acid levels (9.19 ± 0.56% and 9.69 ± 0.43%, respectively), whereas Range-LIM-CORN showed the lowest concentration across all groups (5.49 ± 0.41%), reflecting the influence of corn supplementation on fatty acid profiles. Total n-6 fatty acids (∑ n-6) ranged from 8.53 ± 0.65% in Range-LIM-CORN to 15.62 ± 0.71%

in Range-LIM among range-finished systems, compared with 8.74 ± 0.51% in Pen-FREE-LOW to 10.80 ± 0.77% in pen-finished groups (Table 3). Pen-based finishing, especially under high-density total mixed ration (TMR) feeding (Pen-TMR-HIGH and Pen-TMR-TRAD), was associated with higher n-6:n-3 ratios (5.0–7.3 vs. 2.8–3.6 in range-finished), driven primarily by lower n-3 fatty acid concentrations rather than by higher absolute n-6 levels. In particular, the traditional feedlot system (Pen-TMR-TRAD) exhibited the highest n-6:n-3 ratio (7.25 ± 0.30), with total n-3 concentrations of 1.28 ± 0.04% and linoleic acid at 6.59 ± 0.33%, indicating the most pronounced shift away from favorable fatty acid profiles. In contrast, pen-finished animals fed TMR (Pen-TMR-LOW and Pen-TMR-HIGH) showed intermediate n-6:n-3 ratios of 5.15 ± 0.12 and 5.03 ± 0.11, respectively, with total n-3 concentrations of 1.79 ± 0.12% and 2.13 ± 0.13%, respectively. This likely reflects diets containing proportionally more hay and less grain than the traditional TMR-fed system. Comparisons involving Pen-TMR-TRAD should nevertheless be interpreted cautiously because that treatment originated from a separate breeding herd.

Metabolomic Profiling

In addition to fatty acid profiling, targeted LC-MS/MS analysis of the forages and feed ingredients included 144 phytonutrient metabolites, of which 124 were detected in at least one feed/forage sample. Of the detected compounds, 26 differed significantly among finishing-system feed/forage groups based on one-way ANOVA ($P < 0.05$), indicating marked variation in phenolic metabolite composition across diets. Rangeland forages contained up to 4.2-fold higher summed total phenolic concentrations (329–600 mg/100 g dry weight) than pen-based feeds and supplements (142–262 mg/100 g dry weight). Individual compounds in Table S2 are reported as µg/100 g dry weight, and class totals were calculated as the sum of quantified compounds. Anthocyanins, flavones, and hydroxycinnamic acids were the dominant phenolic classes across the forage and feed samples.

Targeted metabolomic analysis of bison M. longissimus lumborum samples revealed distinct metabolite patterns that were strongly aligned with finishing system and diet composition (Fig. 3). Comprehensive profiling of 43 metabolites in the meat identified 26 compounds that exhibited statistically significant differences across the seven treatment groups (all, $P < 0.05$), with the most pronounced distinctions between range-finished and pen-finished systems (Fig. 3).

Range-finished bison had significantly higher concentrations of phenolic compounds and their microbially derived metabolites (Table S2). Range-DIV exhibited mean hippuric acid concentrations of 74.82 ± 9.97 µg/100 g (mean ± SEM), while Range-LIM exhibited similarly high levels of 79.77 ± 9.17 µg/100 g. Notably, corn supplementation in range-finished animals (Range-LIM-CORN) reduced hippuric acid concentrations to 30.81 ± 4.08 µg/100 g, showing that grain supplementation attenuates the polyphenolic metabolite signature characteristic of forage-based diets. In contrast, pen-finished bison displayed substantially lower concentrations: 16.65 ± 2.04 µg/100 g in Pen-TMR-HIGH, 15.71 ± 1.96 µg/100 g in Pen-TMR-LOW, 22.93 ± 2.82 µg/100 g in Pen-FREE-LOW, and 25.53 ± 2.18 µg/100 g in Pen-TMR-TRAD.

Catechol, another compound derived from plant polyphenols, showed significantly higher concentrations in the range-finished systems ($P < 0.001$). Range-DIV contained 17.21 ± 2.31 µg/100 g catechol, while Range-LIM exhibited similarly high levels of 19.23 ± 2.72 µg/100 g. Pen-finished systems displayed markedly lower concentrations, ranging from 2.99 ± 0.39 µg/100 g (Pen-FREE-LOW) to 7.50 ± 1.09 µg/100 g (Pen-TMR-TRAD) ($P < 0.001$). Corn supplementation reduced catechol concentrations in range-finished animals to 6.99 ± 0.66 µg/100 g.

Shikimic acid, an intermediate in the biosynthesis of aromatic amino acids and plant secondary metabolites, was significantly elevated in the range-finished systems. Range-DIV exhibited 14.76 ± 2.02 µg/100 g, while Range-LIM showed 13.90 ± 1.77 µg/100 g. All pen-finished systems displayed substantially lower concentrations, ranging from 5.52 ± 0.67 µg/100 g (Pen-TMR-LOW) to 6.83 ± 1.12 µg/100 g (Pen-FREE-LOW) ($P < 0.001$). The presence of shikimic acid in meat is consistent with greater exposure to plant-derived metabolites (Table S2).

Conversely, pen-finished bison exhibited elevated concentrations of 3-(4-hydroxyphenyl)lactic acid, a metabolite linked to grain-based feeding and altered microbial-host metabolic interactions. Pen-finished systems showed mean concentrations ranging from 48.12 ± 5.10 µg/100 g (Pen-TMR-TRAD) to 55.65 ± 4.28 µg/100 g (Pen-FREE-LOW), compared with 36.85 ± 4.16 µg/100 g in Range-LIM and 37.31 ± 4.14 µg/100 g in Range-DIV ($P = 0.029$). This metabolite pattern indicates diet-driven shifts in microbial metabolism, with grain-based diets promoting different metabolic pathways than forage-based systems.

Additional metabolites showing significant differences included p-cresol sulfate, which was significantly lower in Pen-TMR-TRAD (61.36 ± 6.37 µg/100 g) than in several other treatments, including Range-LIM (94.83 ± 9.58 µg/100 g) and Pen-TMR-HIGH (93.19 ± 6.10 µg/100 g) ($P = 0.013$). Indoxyl sulfate concentrations were significantly different across groups ($P = 0.033$), with Range-DIV showing 5.18 ± 0.59 µg/100 g compared with 3.18 ± 0.29 µg/100 g in the Range-LIM-CORN system. L-Kynurenine, a tryptophan metabolite, showed significant variation ($P = 0.045$), with concentrations ranging from 3.23 ± 0.51 µg/100 g (corn-supplemented range) to 5.58 ± 0.68 µg/100 g (Pen-TMR-HIGH).

Cinnamoylglycine, a microbially derived metabolite produced from aromatic phenylpropanoid substrates, was significantly elevated in range-finished bison compared with pen-finished animals ($P < 0.0001$). Range-DIV exhibited 0.43 ± 0.07 µg/100 g cinnamoylglycine, while Range-LIM showed 0.37 ± 0.06 µg/100 g. These concentrations were approximately 2-fold higher than the average of the pen-finished groups (0.13 ± 0.03 to 0.19 ± 0.03 µg/100 g for Pen-TMR-LOW and Pen-TMR-HIGH; 0.16 ± 0.04 µg/100 g for Pen-FREE-LOW). The traditional feedlot system (Pen-TMR-TRAD) showed intermediate values (0.33 ± 0.07 µg/100 g), likely reflecting differences in feed composition compared to the other pen-finished treatments. Corn supplementation in range-finished animals (Range-LIM-CORN) reduced cinnamoylglycine concentrations to 0.15 ± 0.02 µg/100 g, which was comparable to pen-finished levels. This metabolite may serve as a potential biomarker associated with aromatic metabolism under forage-rich finishing systems. The relative abundance of these compounds is shown in the hierarchical clustering heatmap (Fig. 4).

Correlation Analysis

Correlation analysis of selected treatment-discriminatory meat metabolites showed that several forage-associated biomarkers were positively associated with one another (Fig. 5). Hippuric acid was positively correlated with catechol ($r = 0.91$, $P < 0.001$), cinnamoylglycine ($r = 0.65$, $P < 0.001$), and shikimic acid ($r = 0.74$, $P < 0.001$), indicating that these metabolites track together across finishing systems. Catechol was also positively correlated with cinnamoylglycine ($r = 0.66$, $P < 0.001$), consistent with shared links to aromatic phenolic metabolism. Together, these relationships support a coordinated meat metabolite signature associated with forage-rich finishing systems, without implying direct transfer of individual compounds from forage to meat.

Treatment-level cross-correlation analysis between fatty acid composition and meat metabolite concentrations revealed strong linkages between tissue n-3 status and forage-associated phenolic biomarkers. Total n-3 fatty acid content was positively correlated with hippuric acid ($r = 0.89$, $P < 0.05$), catechol ($r = 0.82$), and cinnamoylglycine ($r = 0.86$, $P < 0.05$), while the n-6:n-3 ratio was inversely correlated with these same metabolites (hippuric acid: $r = -0.86$, $P < 0.05$; catechol: $r = -0.75$). Alpha-linolenic acid (ALA), the primary plant-derived n-3 fatty acid, showed the strongest positive correlations with catechol ($r = 0.89$, $P < 0.05$) and hippuric acid ($r = 0.86$, $P < 0.05$), consistent with all being indicators of high forage intake. Conversely, linoleic acid and arachidonic acid, the dominant n-6 fatty acids in grain-based diets, showed negative correlations with these forage-associated biomarkers and positive correlations with 3-(4-hydroxyphenyl)lactic acid.

The treatment-level associations in Fig. 6 further underscored the separation between forage- and pen-based systems. Higher forage total phenolics were strongly associated with higher meat hippuric acid (Spearman rho = 0.95, $P < 0.001$; Fig. 6A). Higher forage total phenolics were also associated with higher meat catechol (Spearman rho = 0.77, $P = 0.041$; Fig. 6B), and forage catechol was positively associated with meat catechol (Spearman rho = 0.88, $P = 0.008$; Fig. 6C). Together, these associations suggest that forage phenolic exposure is reflected in the meat biomarker profile rather than indicating direct same-compound transfer.

DISCUSSION

This study found that the finishing system in bison production significantly influences the fatty acid composition and phytonutrient profile of bison meat, with range-finished systems producing meat enriched in n-3 fatty acids and phytonutrients with potential antioxidant and anti-inflammatory properties, compared with bison meat from confinement-based feeding systems. These findings are consistent with previous studies on beef [20], lamb [21], and venison [22], which have found that forage-based diets enhance the omega-3 fatty acid and phytonutrient content in ruminant meat. The results extend the earlier untargeted metabolomics work of van Vliet et al., who reported similar improvements in n-3 fatty acids, phytonutrients, and markers of animal metabolic health in range-finished animals compared to pen-finished animals [13].

The feed and tissue nutrient profiles were broadly aligned across finishing systems, and Fig. 6 highlighted clear associations between forage phenolic composition and meat phenolic biomarkers. The clearest contrast in this study was between range-finished and pen-finished systems, whereas differences attributable to forage diversity within the range-finished systems were comparatively modest given that the limited rangeland still contained an estimated 90 + plant species versus 200 + plant species in the diverse group, arguably making both rangeland treatments quite botanically diverse. Additionally, there were limited differences when the feed in the pen-finished animals was fed as free-choice or as a pre-mixed ration. Because Pen-FREE-LOW provided free-choice access to both hay and corn, the similarity in tissue fatty acid values between Pen-FREE-LOW and Pen-TMR-LOW/Pen-TMR-HIGH is consistent with broadly similar nutrient exposure rather than with differences in feed presentation.

The elevated alpha-linolenic acid (ALA, C18:3n-3) content in range-associated feed profiles (13.10% to 37.95%) compared to pen-based TMR rations (4.53% to 5.04%) likely contributed to higher tissue concentrations of DPA and DHA in the range-finished animals [23, 24]. Notably, EPA (C20:5n-3) concentrations were slightly higher overall in pen-finished bison, with significant differences in some pen-versus-range comparisons, in contrast to the pattern observed for the other long-chain n-3 PUFAs (Table 3; Fig. 2D; Table S1). This pattern may reflect differences in the efficiency of the desaturation-elongation pathway between the feeding systems. In range-finished animals with abundant dietary ALA, the conversion pathway proceeds efficiently through EPA to the longer-chain products DPA and DHA [25], resulting in lower EPA accumulation, as it serves primarily as a metabolic intermediate. Conversely, in grain-fed animals with limited ALA substrate, the reduced flux through this pathway may result in EPA accumulation rather than continued elongation. Additionally, the high linoleic acid content of grain-based diets competes with ALA for Δ6-desaturase, the rate-limiting enzyme in long-chain PUFA synthesis [26], which may further alter the balance of the pathway intermediates. This interpretation is consistent with the substantially higher DPA and DHA concentrations in range-finished bison, indicating that the elongation pathway is likely more complete in range-finished animals than in grain-supplemented animals. These findings highlight the complexity of fatty acid deposition in tissues and suggest that individual fatty acid concentrations should be interpreted within the context of the entire biosynthetic pathway. Range-finished bison exhibited n-6:n-3 ratios between 2.8 and 3.6, which are substantially lower than those of pen-finished animals and approach the ≤ 4:1 ratio recommended for optimal human health [27]. Additionally, feedlot-finished animals fed a traditional TMR exhibited a ratio of 7.25, which was higher than that of other pen-finished animals (5.0 to 5.2). The intermediate n-6:n-3 ratios observed in pen-finished animals (excluding traditional TMR) can be attributed to their diet composition, which contained proportionally more hay and less grain than traditional TMR-fed animals, resulting in a more favorable fatty acid profile that falls between that of range-finished and traditional TMR-fed bison. Western diets typically contain n-6:n-3 ratios ranging from 15:1 to 20:1 [28], which are associated with increased inflammatory responses and cardiometabolic risk [29, 30].

Our findings broadly align with the literature in beef, particularly regarding studies focusing on the enrichment of n-3 fatty acids through dietary modifications in cattle finding higher levels in forage-finished beef [31, 32]. Additionally, a recent profiling study of > 300 samples of grass-fed and grain-fed

beef from commercial North American production systems found that grass-fed beef, particularly those finished on biodiverse pastures, was enriched in n-3 fatty acids compared to grain-fed beef [33]. Importantly, human randomized controlled trials (RCTs) examining fatty acid profiles of ruminant meat have found that consumption of grass-fed meat enriched with n-3 fatty acids can increase circulating n-3 fatty acid concentrations in consumers [7, 34]. These RCTs found that the intake of ruminant meat with enhanced n-3 content leads to measurable increases in plasma and erythrocyte n-3 fatty acid levels in humans [7], reflecting improved bioavailability and incorporation of these beneficial lipids into circulation. This evidence supports the role of ruminant meat as a dietary source capable of modulating circulating fatty acid profiles, which may have downstream effects on health-related biomarkers [35]. Thus, the lower n-6:n-3 ratios in range-finished and pen-finished bison meat with proportionally more forage and less corn than a traditional TMR, suggests that the consumption of such products could contribute to an improved dietary fatty acid balance in human populations; however, our data do not indicate that pen-finished bison meat is inherently unhealthy to consume, particularly given that human intervention studies have not found the same short-term adverse effects for ruminant-derived trans fatty acids as for industrial trans fats in healthy adults [34]. Notably, the n-6:n-3 fatty acid ratio in pen-finished bison was favorable (5.0–5.2), particularly when viewed in the context of the broader literature comparing fatty acid profiles of grass-fed versus grain-fed ruminants with feedlot-finished ruminant meat typically exhibiting ratios of ~ 10:1.

More broadly, diets rich in n-3 fatty acids are consistently associated with improved metabolic health [36, 37]. Omega-3-rich dietary patterns have been linked to reduced systemic inflammation, improved lipid profiles [38], enhanced insulin sensitivity [39], and favorable modulation of cardiovascular risk factors [40]. These effects are thought to arise from their capacity to influence cell membrane fluidity [41], metabolism-related gene expression [42], and anti-inflammatory signaling pathways [43]. For example, higher dietary n-3 intake and lower n-6:n-3 ratios have been linked to reduced systemic inflammation [44], as evidenced by lower concentrations of inflammatory markers such as C-reactive protein, interleukin-6, and tumor necrosis factor-alpha [45].

Phytonutrient profiling revealed that the finishing system also influences the broader metabolite signature of bison meat. The metabolite patterns observed in this study reflect the fundamental differences in dietary inputs and their biotransformation through ruminal and host metabolism [46]. Range-finished animals consuming a diverse array of forages ingest a wide array of plant secondary metabolites, including flavonoids, phenolic acids, and other polyphenolic compounds. Targeted phenolic profiling of the forages used in this study confirmed this pattern: rangeland forages contained summed total phenolic concentrations of 329–600 mg/100 g dry weight compared with 142–262 mg/100 g dry weight in pen-based feeds (Table S2). These totals were calculated from the individual compounds reported in Table S2 as µg/100 g dry weight. Notably, the feed corn sample contained the lowest summed total phenolics (53 mg/100 g dry weight), consistent with the attenuated phenolic biomarker profile observed in corn-supplemented systems[14].

Forage phytonutrients may be deposited more directly in tissue or transformed by the ruminal microbiota, yielding metabolites such as hippuric acid (from benzoic acid and other aromatic precursors) and catechol (from plant phenolics), whereas shikimic acid more directly reflects plant aromatic biosynthesis pathways [47]. Thus, the enrichment of hippuric acid, catechol, and cinnamoylglycine in range-finished animals reflects the consumption and microbial transformation of plant polyphenols, which are abundant in diverse pasture systems but are lower in grain-based concentrates [48]. In particular, hippuric acid is formed through the conjugation of benzoic acid, derived from dietary polyphenols, with glycine in the liver [49]. In non-uremic humans, higher levels of hippuric acid are associated with improved gut microbial diversity and a lower risk of metabolic syndromes [50, 51]. In ruminants, hippuric acid is considered a potential biomarker of grass-finishing [52] and has also been discussed in relation to animal metabolic health [14].

Cinnamoylglycine was elevated ~ 2–3-fold in the range-finished bison compared to the pen-finished bison. Cinnamoylglycine is a microbially derived metabolite formed from cinnamic acid-related aromatics and subsequently conjugated with glycine in the liver [53]. Higher forage intake increases ruminant exposure to phenylpropanoids and other aromatic substrates, resulting in elevated levels of cinnamoylglycine, which can potentially also serve as a biomarker of forage-driven aromatic metabolism [54]. These findings are in line with previous work on bison, which found that cinnamoylglycine was ~ 3-fold higher in range-finished than in pen-finished bison on feed [13]. In ruminants, this pattern is generally interpreted as indicating greater phenolic exposure and has been linked, in broader pasture/plant-rich dietary contexts, to metabolic signatures consistent with improved animal metabolic health (e.g., oxidative stress and mitochondrial function pathways) [15]. In humans, cinnamoylglycine is correlated with diet quality and is inversely associated with the risk of type 2 diabetes [55]. Whether dietary exposure to cinnamoylglycine through animal food directly impacts circulating levels in humans is currently unknown. Importantly, forage-derived phytochemicals may help limit lipid oxidation in meat, but whether this meaningfully affects downstream circulating n-3 fatty acid levels in consumers remains unclear [56, 57].

In contrast, pen-finished systems relying on grain-based total mixed rations (TMR) or free-choice grain supplementation produce different metabolic signatures. Elevated concentrations of 3-(4-hydroxyphenyl)lactic acid in pen-finished bison represent a metabolite signature associated with grain-based feeding and altered ruminal fermentation patterns [13]. This metabolite is produced through the microbial metabolism of tyrosine and related aromatic amino acids under conditions typical of high-grain diets, which favor different microbial populations than those in forage-based systems. This comparison also shows how energy supplementation on a low-diversity pasture compares with a high-diversity pasture without supplementation [58, 59]. The inclusion of corn supplementation on rangelands resulted in intermediate fatty acid profiles, with n-3 concentrations lower than those in exclusively range-finished bison but higher than those in the pen-finished groups. Additionally, hippuric acid showed a more intermediate profile, with levels lower than those in bison finished exclusively on forages but higher than those in bison finished on grain-based concentrates. This finding indicates that, even within forage-based systems, dietary composition exerts measurable effects on meat nutritional composition (Table

S2). Producers seeking to optimize meat nutritional composition while maintaining some degree of supplementation should consider the trade-offs between animal performance and nutritional attributes when formulating finishing diets. Additionally, producers can consider feeding grains on pastures during limited times of the year (e.g., during the winter) to ensure adequate energy intake as opposed to using it as a finishing ration. Furthermore, while high stocking densities can increase animal stress, which may alter metabolism [60], our results indicated no significant differences in meat fatty acid or metabolite profiles between the high and low stocking density pen systems.

The clear separation between range- and pen-based systems in the hierarchical clustering analysis (Fig. 4) indicates that metabolomic profiling can effectively authenticate production methods and distinguish meat from different finishing systems, as has also been shown in beef and goat milk products [61, 62]. The ability to distinguish between range- and pen-finished systems and detect the effects of corn supplementation further highlights the potential of metabolomics as a tool for verifying production claims and understanding the biochemical consequences of different management strategies. These findings add to the growing body of evidence that finishing practices fundamentally alter the metabolite composition of meat, with implications for meat quality, and potential animal and human health outcomes [63], although the latter requires further study attention.

The correlation analyses further highlight the integrated nature of the nutritional differences between finishing systems. The strong intercorrelations among hippuric acid, catechol, and cinnamoylglycine suggest that these metabolites rise together across finishing systems and are likely influenced by both ruminal and host metabolism (Fig. 5). Together with the treatment-level forage-to-meat associations (Fig. 6), these patterns reinforce the separation between forage- and grain-associated metabolic signatures. Importantly, the cross-correlation between fatty acid profiles and metabolite concentrations indicates that these two dimensions of meat composition are not independent but rather co-vary as a function of dietary intake. Range-finished animals with favorable n-3 fatty acid profiles also carried higher concentrations of phytonutrient-related metabolites (Figs. 2, 3, 5, and 6), whereas pen-finished animals with elevated n-6:n-3 ratios showed lower levels across many phytonutrient metabolites. From a consumer perspective, these findings suggest that range-finished bison meat differs from pen-finished meat not only in n-3 enrichment but also in a broader set of metabolites associated with forage intake and polyphenolic metabolism. These integrated profiles are consistent with broader literature linking higher n-3 and phytochemical intakes with reduced inflammation and improved cardiometabolic health outcomes [64], although those effects were not directly tested here.

Integrating sensory and chemical signatures

To connect these production-driven chemical differences to eating quality, we related the meat metabolite and fatty-acid gradients measured here to trained descriptive-panel sensory scores reported for the same six diverse finishing systems [8] (Fig. 7). Sensory attributes that were most intense in range- or forage-finished steaks (green-hay, liver-like, musty or earthy, sour-aromatic, and overall bison-identity character) were positively correlated across systems with the plant-derived phenolics (hippuric

acid, catechol, shikimic acid, cinnamoylglycine) and long-chain omega-3 fatty acids that were likewise elevated in range-finished bison (Spearman ρ up to 0.94). Conversely, attributes that were most intense in pen- or TMR-finished steaks (juiciness, tenderness, overall sweetness, umami, and bloody or serum character) were positively correlated with palmitic acid and the n-6:n-3 ratio and negatively correlated with the phenolic biomarkers. This helps explain the perception that forage-finished bison carries a grassy or pasture character, while grain- or TMR-finished bison is perceived as juicier, fattier, and more umami [65–68]. For producers, this links consumer preference to a production decision: finishing system can be matched deliberately to a target flavor profile and market segment. Because these correlations are based on six treatment-mean values, they are interpreted as illustrative associations rather than causal proof and warrant confirmation in integrated sensory–metabolomic studies.

From a sustainability perspective, the findings suggest that well-managed range-finished systems can produce meat with enhanced nutritional profiles while potentially supporting ecosystem function through the maintenance of native grasslands and promotion of biodiversity, as well as associated ecosystem services [69]. The diverse plant communities in range-finished systems may enhance the phytochemical content of meat while also contributing to soil health, carbon sequestration, and habitat provision [14, 70].

This study has several limitations. First, the conventional TMR feedlot treatment originated from a separate breeding herd, so differences involving Pen-TMR-TRAD cannot be attributed exclusively to finishing system. Second, while the limited diversity rangeland treatment was more limited in plant diversity compared to the diverse rangeland, the "limited" diversity rangeland still contained approximately 90 plant species. Although it was dominated by a few meadow grasses, it was already highly diverse and in far excess of the diversity found in many pastures. This high baseline diversity explains why the metabolomics and phytonutrient profiles for the "diverse" forage group did not consistently exceed, and in some respects were even less than, those of the "limited" group. As a result, the "limited" range treatment here should not be interpreted as a typical low-diversity pasture dominated by a few species of plants. Third, this study did not directly evaluate consumer sensory preferences or willingness to pay for meat from different finishing systems, both of which are important factors for market viability. However, carcass characteristics, proximate composition, and palatability outcomes from these same finishing systems were reported previously by O'Sullivan et al. (2025). Finally, while this study investigated phytonutrient and fatty acid profiles, it is possible that broader nutritional differences exist between treatment groups in terms of minerals, vitamins, and protein content; however, the focus in this work was on fatty acids and phytonutrients as they tend to provide the greatest contrast between forage and grain-based finishing systems [71].

CONCLUSIONS

This study found that finishing environment is a primary determinant of the fatty acid and phytonutrient composition of bison meat, although comparisons involving the conventional TMR feedlot treatment should be interpreted cautiously because that group originated from a separate breeding herd. Range-

finished systems enhanced n-3 polyunsaturated fatty acids and polyphenol-derived metabolites, whereas the conventional TMR feedlot treatment produced the highest n-6:n-3 ratios and the lowest phytochemical content, while corn supplementation on pasture yielded an intermediate profile. These findings do not suggest that pen-finished bison meat is unhealthy, but they do indicate that range-finished bison meat offers a more favorable biochemical profile consistent with dietary patterns linked to improved cardiometabolic health. Linking these chemical gradients to trained descriptive sensory-panel scores from the same finishing systems further indicated that the forage-associated phenolic and n-3 fatty-acid signatures align with the characteristic green, earthy flavor of range-finished bison, whereas pen-finishing aligned with juiciness, tenderness, and umami attributes, offering producers a practical means to match finishing strategy to a target flavor profile and market segment. Future research should translate these compositional differences into measurable health outcomes through human feeding trials, confirm these chemical–sensory associations in integrated sensory–metabolomic studies, and evaluate the economic feasibility of range-finishing systems.

Abbreviations

ADF
acid detergent fiber
ALA
alpha-linolenic acid
CLA
conjugated linoleic acid
CP
crude protein
DHA
docosahexaenoic acid
DM
dry matter
DPA
docosapentaenoic acid
EPA
eicosapentaenoic acid
FAME
fatty acid methyl ester
GC-FID
gas chromatography–flame ionization detection
LA
linoleic acid
LC–MS/MS
liquid chromatography–tandem mass spectrometry

MUFA
monounsaturated fatty acid
NDF
neutral detergent fiber
NEm
net energy for maintenance
PUFA
polyunsaturated fatty acid
QC
quality control
SARE
Sustainable Agriculture Research and Education
SEM
standard error of the mean
SFA
saturated fatty acid
TDN
total digestible nutrients
TMR
total mixed ration
USDA
United States Department of Agriculture.

Declarations

Ethics approval and consent to participate

All bison management and harvest procedures adhered to the guidelines and regulations established by the United States Department of Agriculture. Because the study used animals finished under standard commercial ranching conditions with no experimental intervention or handling by the research team, Institutional Animal Care and Use Committee approval was not required for this study.

Consent for publication

Not applicable.

Competing Interests

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projects that link agricultural production systems to the nutritional/metabolite composition of animal and plant foods. SvV has recently received consulting fees from Perdue Foods and Big Bold Health. SvV also reports travel honoraria and speaker fees for presentations related to their research. SvV is a non-paid member of the Scientific Advisory Committee of the Food and Agriculture Organization of the United Nations. C.K. is a paid employee of the Turner Ranches. The remaining authors declare that they have no competing interests that could influence the work reported in this paper.

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Author Contribution

J.V.V. performed formal analysis, investigation, data curation, visualization, and wrote the original draft, with writing review and editing. A.B. and G.W. managed animal production, sample collection, and carcass fabrication. L.O'S. assisted with feeding and management data organization. J.V.V., M.A., Y.X., and R.W. performed laboratory analyses, including fatty acid and metabolomic profiling. S.v.V., A.B., G.W., and R.W. provided critical revisions and interpretations of the results. S.v.V. contributed conceptualization, methodology, validation, investigation, resources, supervision, project administration, funding acquisition, and writing review and editing. C.K. contributed conceptualization, study design, resources, funding acquisition, and writing review and editing. All authors reviewed and approved the final manuscript.

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Data Availability

All data generated or analyzed during this study are included in this published article and its supplementary information files. Fatty acid composition data are provided in Supplementary Table S1, and individual plant and meat metabolite concentrations are provided in Supplementary Table S2.

Additional datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

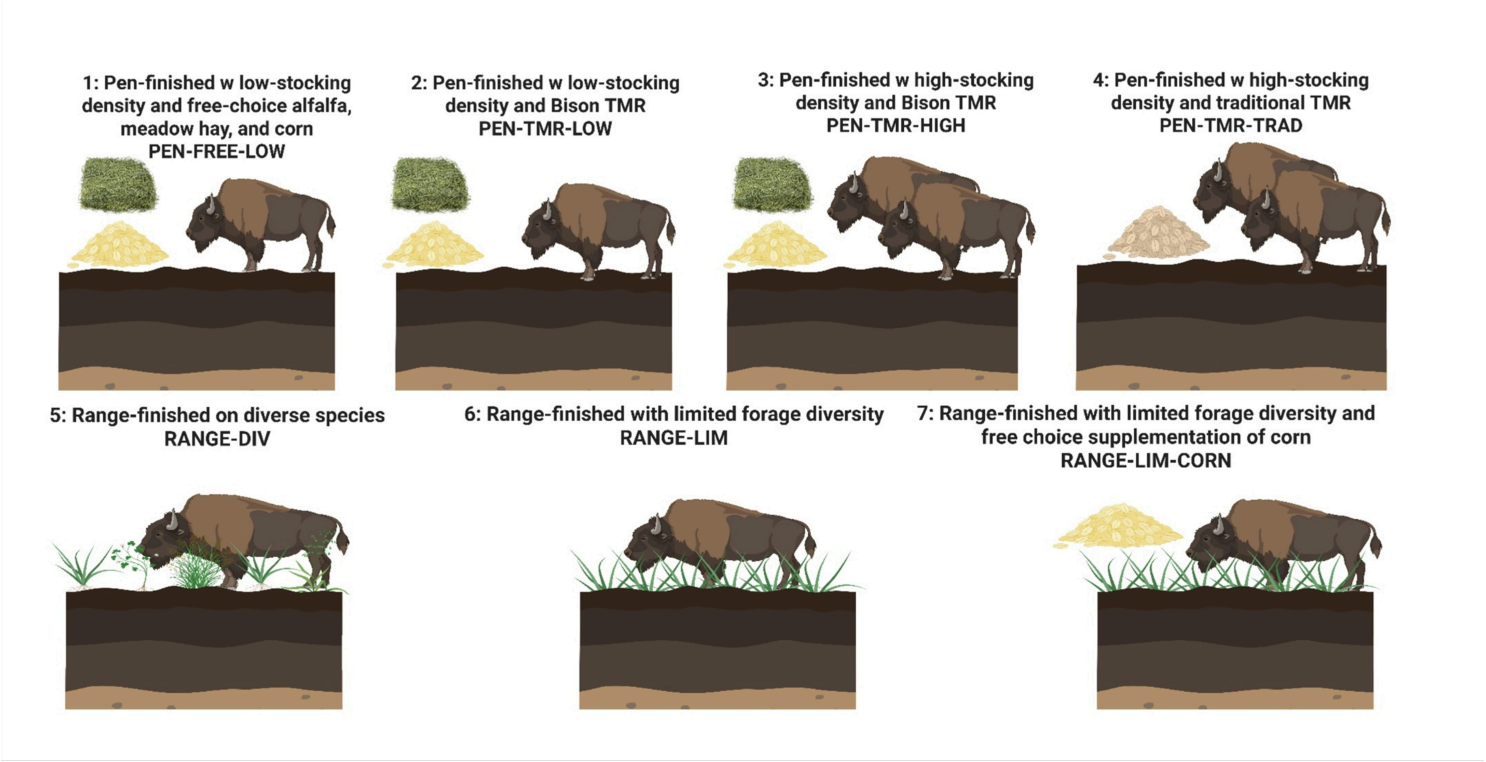


Figure 1

Overview of the bison finishing systems and study design.

Bison heifers were finished under seven production systems differing in diet composition, forage diversity, pasture versus pen-based management, and stocking density. Pen-based systems included free-choice hay/corn feeding and total mixed ration (TMR) systems under low- and high-density conditions, including a traditional feedlot-style TMR system. Range-based systems included a botanically diverse native rangeland, a lower-diversity meadow pasture, and a lower-diversity pasture supplemented with corn. Following the finishing period, striploin samples (M. longissimus lumborum) were collected for fatty acid and targeted phytonutrient metabolomic analyses. Created with BioRender.

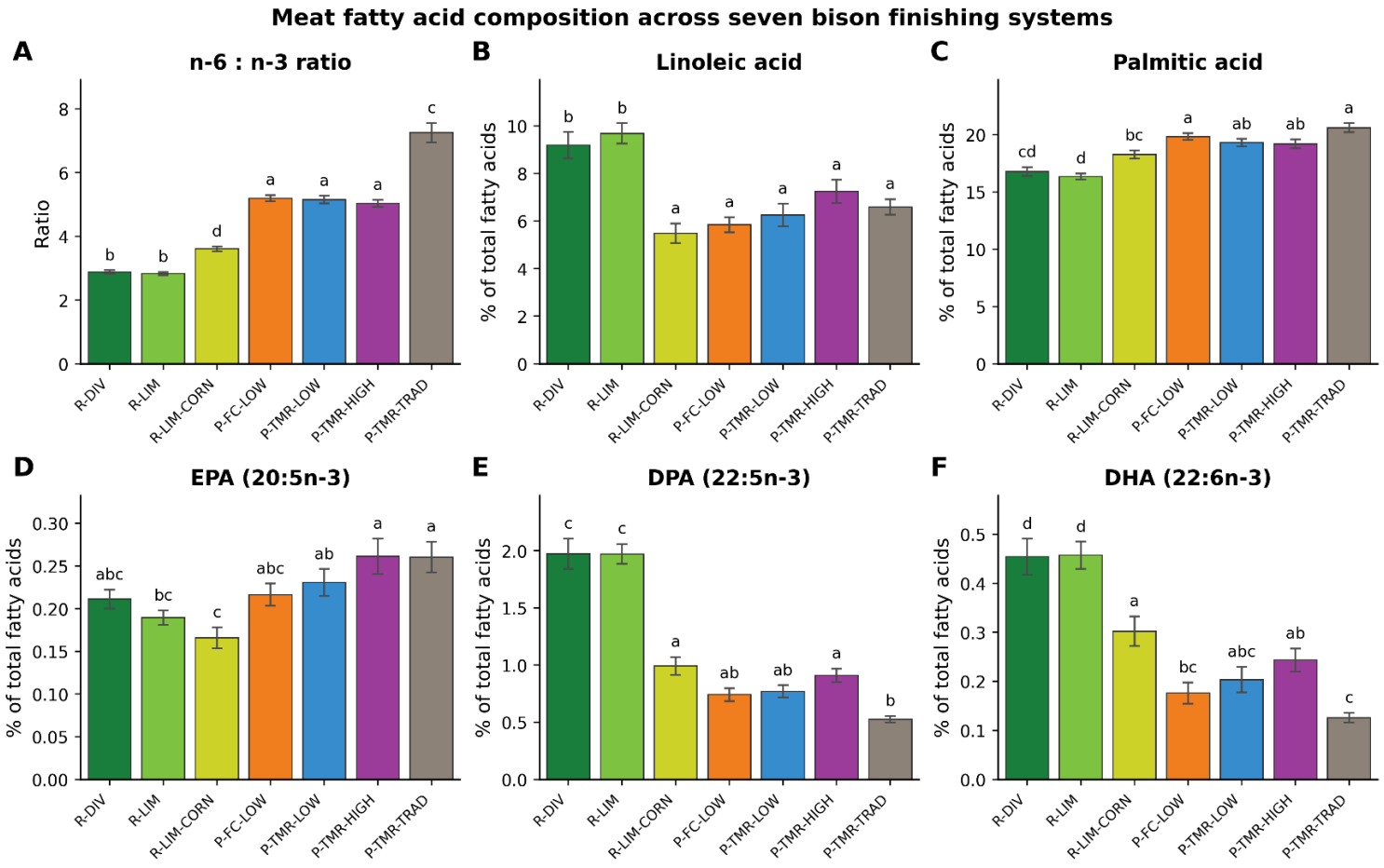


Figure 2

Fatty acid composition of bison striploins across seven finishing systems. Fatty acid composition of M. longissimus lumborum from bison finished under seven production systems. Panels show (A) n-6:n-3 ratio, (B) linoleic acid (LA; C18:2n-6), (C) palmitic acid (C16:0), (D) eicosapentaenoic acid (EPA; C20:5n-3), (E) docosapentaenoic acid (DPA; C22:5n-3), and (F) docosahexaenoic acid (DHA; C22:6n-3). Values are expressed as % of total identified fatty acids and presented as mean ± SEM (n = 16 per treatment). Different letters indicate significant differences among finishing systems at $P < 0.05$ according to Tukey's multiple-comparisons test.

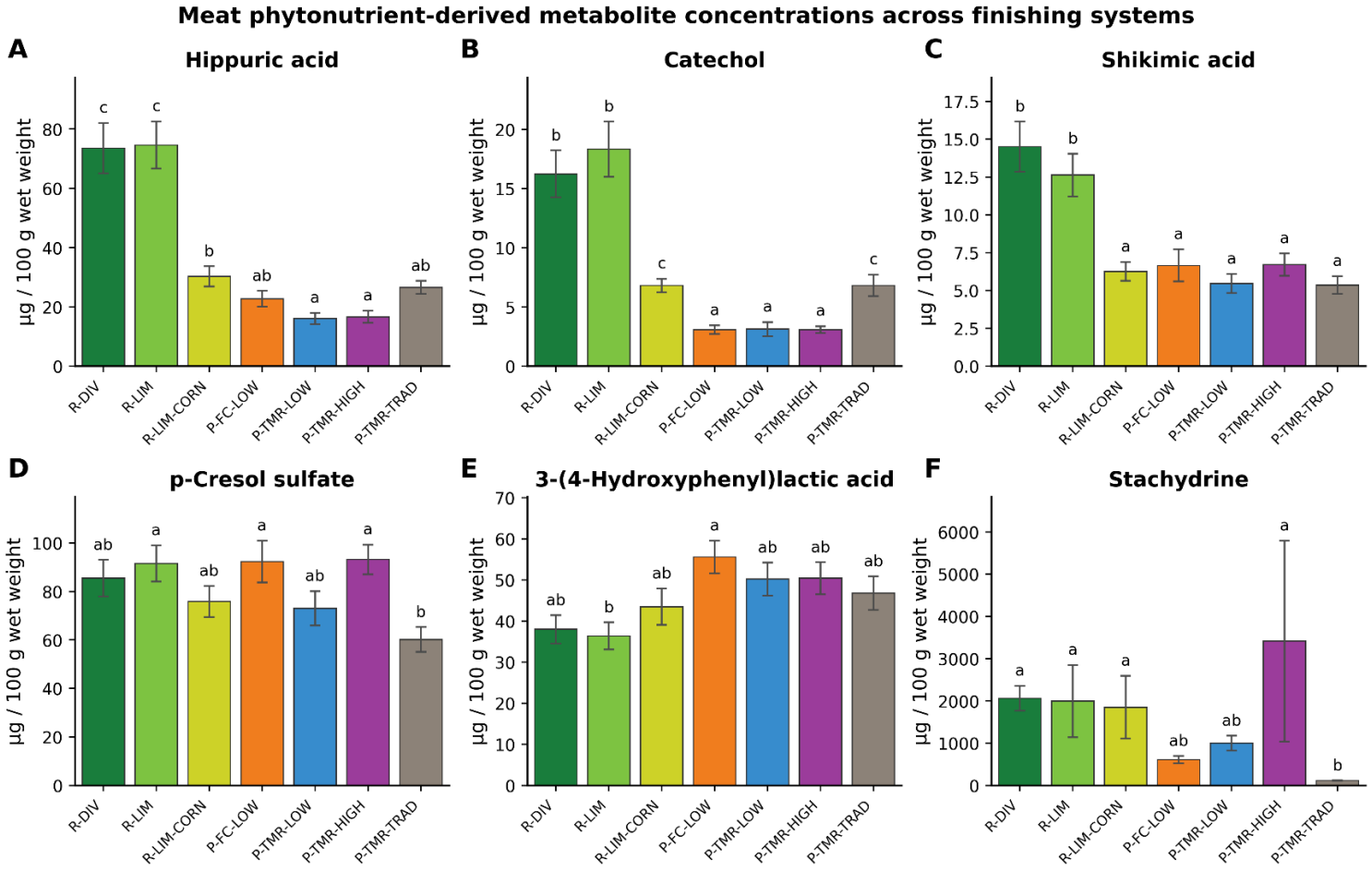


Figure 3

Targeted phytonutrient-derived metabolite concentrations in bison striploins across seven finishing systems. Targeted metabolomic profiling of M. longissimus lumborum from bison finished under seven production systems. Panels show concentrations of (A) hippuric acid, (B) catechol, (C) shikimic acid, (D) p-cresol sulfate, (E) 3-(4-hydroxyphenyl)lactic acid, and (F) stachydrine. Values are expressed as µg/100 g wet weight and presented as mean ± SEM (n = 16 per treatment). Different letters indicate significant differences among finishing systems at $P < 0.05$ according to Tukey's multiple-comparisons test.

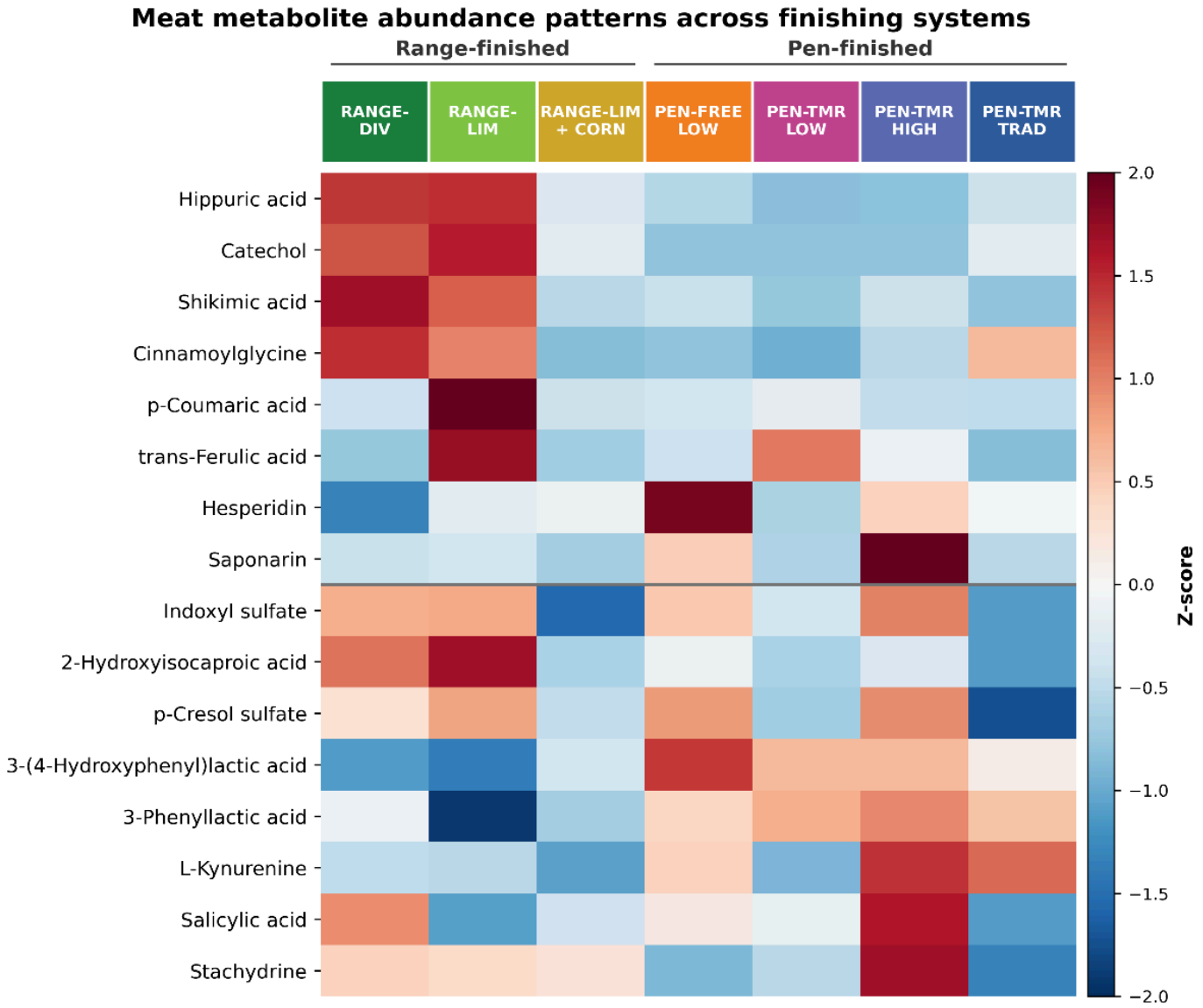


Figure 4

Hierarchical clustering heatmap of targeted metabolite concentrations in bison striploins across seven finishing systems. Hierarchical clustering heatmap showing the relative abundance of targeted metabolites in M. longissimus lumborum from bison finished under seven production systems. Metabolite concentrations are displayed as row-scaled Z-scores. Columns represent treatment groups, organized by finishing system, and rows represent individual metabolites. Red indicates relatively higher abundance and blue indicates relatively lower abundance. This analysis illustrates the separation of range-finished and pen-finished systems based on their metabolomic signatures.

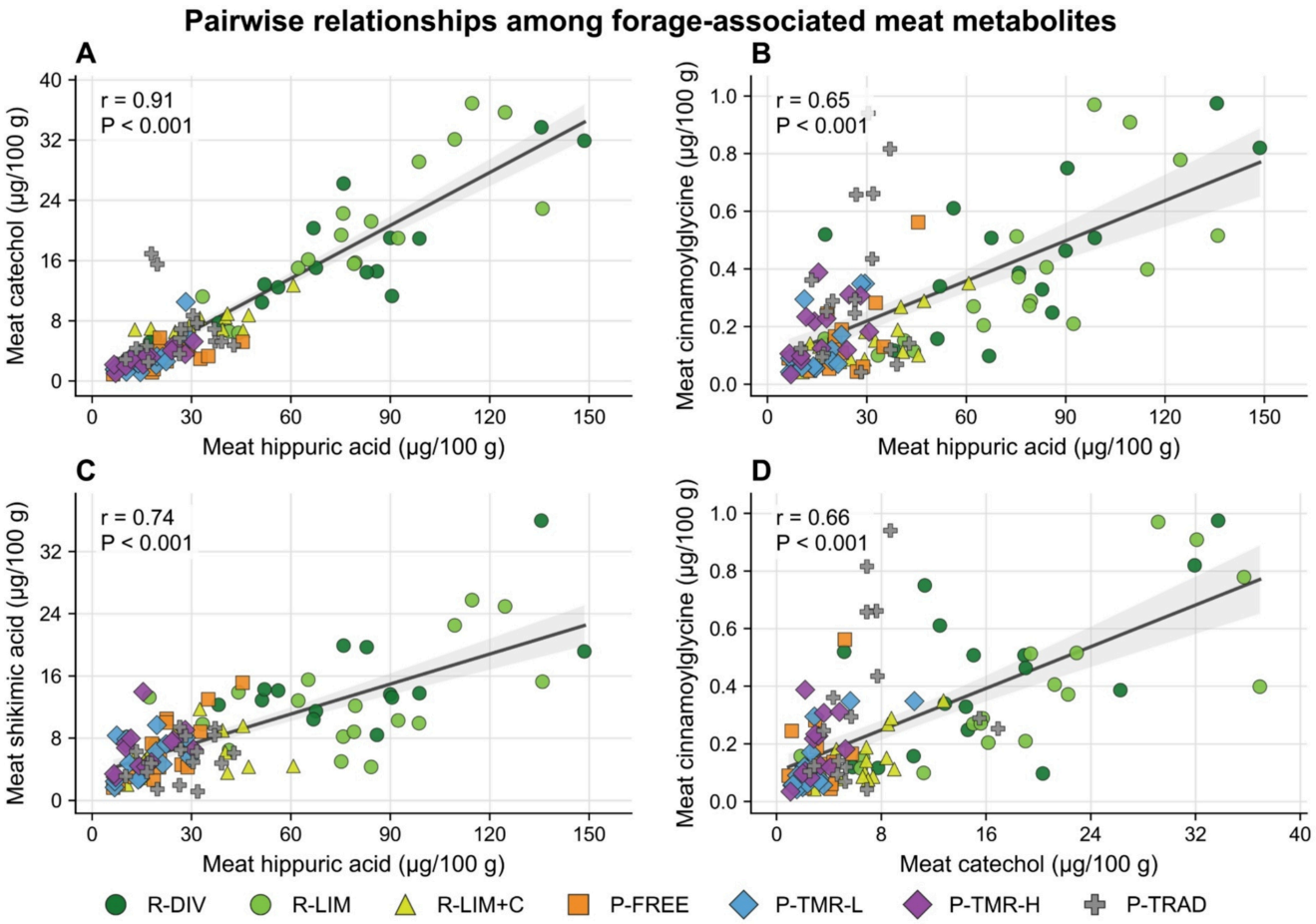


Figure 5

Relationships among selected forage-associated meat metabolites in bison longissimus muscle. Scatterplots show pairwise associations among treatment-discriminatory meat metabolites across finishing systems. Panels show relationships between (A) hippuric acid and catechol, (B) hippuric acid and cinnamoylglycine, (C) hippuric acid and shikimic acid, and (D) catechol and cinnamoylglycine. Points represent individual meat samples and are colored by finishing system. Solid lines indicate fitted linear relationships. Correlation coefficients and P values are shown within each panel.

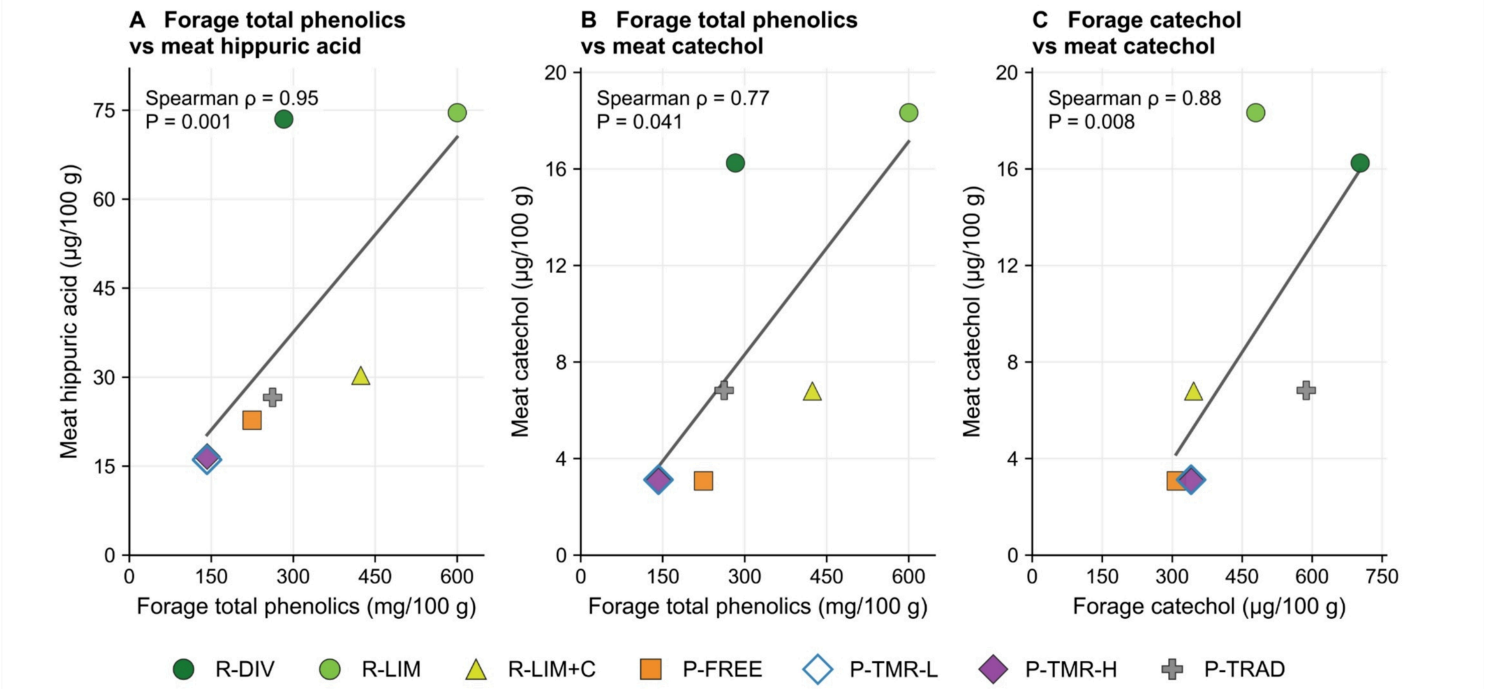


Figure 6

Treatment-level associations between forage phenolic composition and meat phenolic biomarkers across finishing systems. Relationships between treatment-mean forage and meat values across the seven finishing systems. Panels show associations between (A) forage total phenolics (mg/100 g dry weight) and meat hippuric acid, (B) forage total phenolics and meat catechol, and (C) forage catechol and meat catechol. Each point represents the treatment mean for one finishing system. Spearman rho values and corresponding P values are shown within each panel. These associations are interpreted as biomarker relationships rather than direct same-compound transfer.

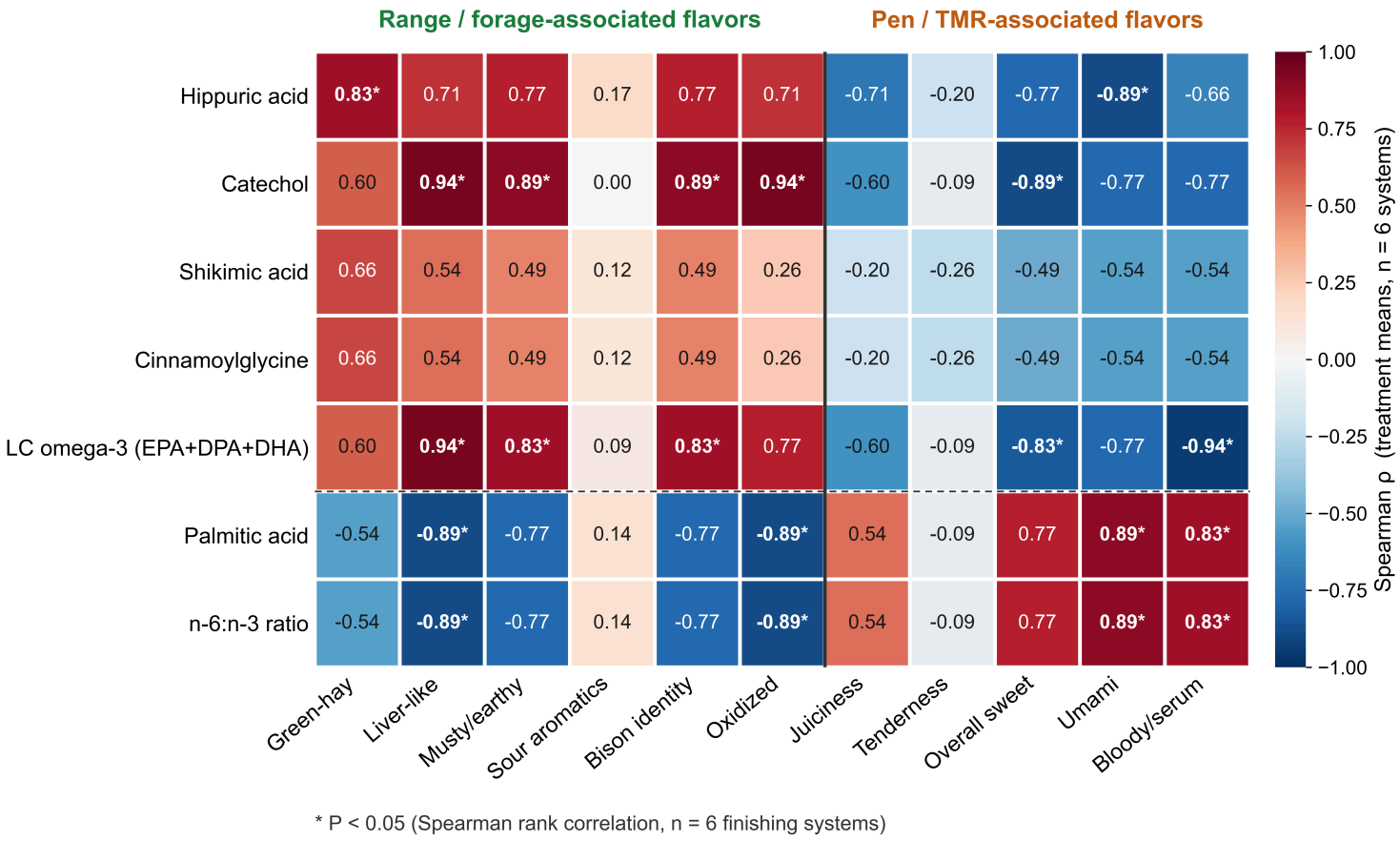


Figure 7

*Treatment-level Spearman correlations (ρ) between trained descriptive-panel sensory attributes and meat chemistry across the six diverse bison finishing systems common to both datasets (range-finished: R-DIV, R-LIM, R-LIM+C; pen-finished: P-FREE, P-TMR-L, P-TMR-H; n = 6 system means). Sensory least-squares means are from a trained panel evaluating 14-d aged striploin steaks; meat phenolic metabolites and fatty acids are from the present study. Rows progress from range- / forage-associated chemistry (plant phenolics, long-chain omega-3) to pen- / TMR-associated chemistry (palmitic acid, n-6:n-3 ratio); columns group forage-associated flavors (left) and pen / TMR-associated flavors (right). Red denotes positive and blue negative correlation; * P < 0.05. Forage-associated flavors track phenolic / omega-3 enrichment, whereas juicy, fatty, and umami attributes track the fat-associated chemistry of grain / TMR finishing.*