



The effects of including sprouted barley in the diets of angus finishing steers on meat quality, sensory analysis, and meat metabolome

Zachary Cal Crump[†], Audrey Donna Lidgard[‡], Chandler Douglas Stafford[‡], Taelie Ann Nauvoo Kennedy[†], Anthony Fernando Alberto[†], Bailee Lynn Brown[†], Bryce Wayne Roholt[†], Lillian Lelehua Okamoto[†], Joseph Vinod Varre[‡], Lamis Ali[‡], Muhammad Ahsin[‡], Ryan Arthur Spurling[†], Brady Morris Blackett^{||}, Robert Lynn Harding^{||}, Sanjeeva Dilshan Ranathunga^{||}, Stephan Van Vliet[†], Sulaiman Khalid Matarneh[‡], and Kara Jean Thornton^{†,1}

[†]Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, UT 84322

[‡]Department of Nutrition, Dietetics, and Food Sciences, Utah State University, Logan, UT 84322

^{||}Renaissance Ag, Vineyard, UT 84057

¹Corresponding author: kara.thornton@usu.edu

Abstract

Improving sustainability of agricultural production has been at the forefront of research in recent years due to external factors, such as drought and urbanization. Producers are interested in exploring new practices to remain viable. One practice being researched is vertical farming systems (VFS) to produce feed. These VFS control temperature, water, light, and sprout cereal grains that can be fed whole to livestock, but to date, little research has been completed on feeding sprouted grains to livestock. This study utilized growing Angus steers ($n=60$) that were stratified by weight ($385 \text{ kg} \pm 10.3$) into two different diet groups, control (CON, $n=30$) or sprouted barley (SB, $n=30$). The CON diet was a traditional finishing ration for the region (rolled barley, corn silage, and alfalfa), while SB was fed a ration with 20% dry matter (DM) sprouted barley. All animals were fed out of Vytelle[®] units to assess individual intake. At harvest, one loin from each animal was obtained for meat quality analysis. Meat quality was assessed using PROC MIXED in SAS, with day as a repeated measure to evaluate main effects of diet on color. Sensory data were analyzed using a paired two-way t-test. Welch's t-test was employed for meat metabolome analysis, while feed metabolome data was evaluated using an ANOVA. Color (L^* , a^* , and b^*) nor three meat quality markers (cooking loss, drip loss, Warner-Bratzler Shear Force) were impacted by diet ($P \geq 0.19$). Deoxymyoglobin (DMb) content was not affected by diet ($P=0.18$), but metmyoglobin (MMb) was ($P=0.05$) and oxymyoglobin (OMb) tended ($P=0.08$) to be different. MMb increased over time for both diets, but CON generally had increased MMb compared to SB. Changes in OMb were explained by both CON and SB decreasing over the seven-day period with SB generally having more OMb compared to CON throughout. A consumer acceptance panel revealed SB and CON to not be different ($P \geq 0.11$) in terms of liking for overall acceptance, aroma, flavor, tenderness, nor juiciness. Despite 71 out of 85 phytochemical metabolites being different in the feeds ($P < 0.05$) with 28 being elevated in the SB feed at 20% DM, only two out of 23 phytochemical metabolites differed ($P < 0.05$) in meat samples. The inclusion of sprouted barley at 20% DM in the diets of finishing Angus steers had no significant impact on meat quality, nor many aspects of sensory analysis.

Lay Summary

Traditional farming practices are strained by drought and urbanization. As a result, researchers are exploring sustainable alternatives, including vertical farming systems. These systems control light, water, and temperature to sprout cereal grains, like barley, which can then be fed to livestock. In this study, 60 Angus steers were divided into two groups: one fed a traditional finishing diet and the other fed a diet containing 20% sprouted barley (dry matter). After harvest, meat quality was assessed based on color, tenderness, flavor, and metabolomic profile. Results showed that including sprouted barley had no significant effect on meat quality or taste. Although the sprouted barley feed contained more diverse plant compounds compared to the traditional feed, few of these differences carried over into the meat. Overall, a 20% inclusion of sprouted barley in the diet had no measurable impact on meat quality. This suggests that increased inclusion rates may be necessary to observe meaningful differences. Feeding sprouted barley at 20% offers an alternative option for producers without negatively impacting production.

Key words: beef cattle, meat quality, phytochemicals, sprouted barley, vertical agriculture

Abbreviations: ADF, Acid detergent fiber; CON, Control; DM, Dry matter; DMb, Deoxymyoglobin; MMb, Metmyoglobin; MTBE: MeOH, methyl tert-butyl ether: methanol; NDF, Neutral detergent fiber; NE_m , Net energy maintenance; NE_g , Net energy gain; OMb, Oxymyoglobin; PSE, Pale soft exudative; PTFE, polytetrafluoroethylene; SB, Sprouted barley; VFS, Vertical farming system; WBSF, Warner-Bratzler shear force; WHC, Water holding capacity

Introduction

Improving beef production is crucial for livestock producers to meet the demands of an increasing global population and

growing demand for beef (Alexandratos and Bruinsma, 2012). Beef producers face new challenges due to increased urbanization of arable land and climate variability, such as drought

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(Countryman et al., 2016). In particular, the Western United States experienced its worst drought conditions in the last 1,200 years between 2000 and 2018 (Leeper et al., 2022). Additionally, urbanization of arable land has negatively impacted operators' ability to produce consistent and quality livestock feed. It was estimated that 48 million acres of arable land were lost between 2002 and 2022 the U.S. (Agriculture, 2023). These factors influence input costs for beef production and the economic viability of an operation.

Vertical farming systems (VFS) are being used with increasing popularity to produce consistent feed, such as sprouted grains, using decreased land and operating independently of inclement weather (Ahamed et al., 2023). These VFS control factors such as temperature, humidity, water, and light, providing producers with greater control over production. While using VFS, a five-to-six-inch sprout can be produced in six to seven d and then fed whole, as a forage, to livestock. These sprouts may also influence meat quality, as finishing diets such as grass-fed and grain-fed cattle have been shown to affect consumer preferences (Duarte et al., 2022), highlighting the need to examine how feeds produced in VFS impact meat quality and consumer acceptance (Morales Gómez et al., 2022). Cattle diets containing more forages can result in leaner carcasses compared to cattle fed concentrate-based diets (Nogoy et al., 2022). However, others report no carcass differences when replacing alfalfa haylage with corn silage (Tarnonsky et al., 2023). Forages typically contain more phytochemicals than typical feedstuffs, such as corn, which can further impact flavor and quality of meat (van Vliet et al., 2021). Many of these compounds are metabolized and deposited into the muscle, therefore, metabolomic profiling provides a powerful tool to link dietary phytochemicals with changes in meat composition, flavor, and potential health-promoting attributes (Vasta and Luciano, 2011; Provenza et al., 2019; van Vliet et al., 2021).

The goal of this study was to determine how sprouted barley grown in a VFS could impact meat quality, consumer acceptance, and the metabolomic profile of the meat when included in the diets of finishing Angus steers. It is hypothesized that incorporating sprouted barley into the diets of Angus steers would not affect meat quality or consumer preference, but the phytonutrient concentrations in the meat would increase compared to conventional diets.

Methods

Animal care and feeding

The animal portion of this study was conducted at the Utah State University (USU) South Farm (Wellsville, Utah) and was approved by IACUC #13709. Sixty angus-influenced steers ($n=60$) were stratified by weight ($385\text{ kg} \pm 10.3$) into two different diet groups ($n=30$). All steers were implanted with Ralgro® (Merck Animal Health, Madison NJ, USA) at the beginning of the study and were fed for approximately 130 d. One diet consisted of a typical finishing ration for the Intermountain West ($n=30$; CON), while the other included 20% dry matter (DM) sprouted barley ($n=30$; SB) replacing corn silage and some rolled barley. Diets were mixed in a vertical mixer, delivered to animals twice daily, and were fed *ad libitum*. Pens were equipped with Vytelle (Vytelle, Calgary, Alberta, Canada) feed bunk units to measure individual animal intake.

The nutritional analysis of each of the diets can be seen in Table 1. Feedlot performance and carcass traits can be seen in Table 2. When animals reached an industry standard of 12 mm of 12th rib fat thickness with a BW of $595.88\text{ kg} \pm 17.36$ steers were commercially harvested (JBS, Hyrum, Utah, USA). During fabrication, the right loin of each animal was tagged and pulled from the fabrication line 48 h postmortem then immediately vacuum packaged and were allowed to wet age for 12 d at 4 °C. After wet aging for 12 d, a section (approximately three, 2-cm steaks) was portioned, vacuum packaged, and stored under refrigeration for an additional four d before being used in the sensory panel to accommodate logistical scheduling. The remaining section of the loin was fabricated into two cm steaks and randomly allocated for either surface color and myoglobin form evaluation or other meat quality analyses (pH, drip loss, and Warner–Bratzler shear force (WBSF)). Steaks for pH, drip loss, and WBSF were evaluated immediately following the 12 d wet aging period. Steaks were never frozen at any point prior to sensory or meat quality analysis.

Table 1. Nutritional analysis of diets fed to Angus finishing steers

Diet composition, % DM	CON ¹	SB ²
Alfalfa	8.5	8.5
Corn Silage	8.7	0
Rolled Barley	80.5	67.9
Sprouted Barley	0	21.3
Finishing Mineral	2.3	2.3
Nutrient Composition ³ , DM		
DM, %	78.3	34.9
CP, %	13.3	15.8
NE _m , Mcal/lb	0.88	0.89
NE _g , Mcal/lb	0.59	0.60
NDF, %	23.4	21.9
ADF, %	14.5	14.1
Ash, %	5.5	5.9

¹CON, control diet;

²SB, 20% inclusion of sprouted barley in the diet;

³Dry matter (DM); crude protein (CP); net energy maintenance (NE_m); net energy gain (NE_g); neutral detergent fiber (NDF); acid detergent fiber (ADF).

Table 2. Average feedlot performance and carcass characteristics from feedlot trial

	CON ¹	SB ²	SEM	P-Value
DMI ³ , kg	11.27	8.48	0.32	<0.0001 [†]
ADG ⁴ , kg	1.69	1.60	0.22	0.54
G: F ⁵	0.15	0.19	0.01	0.006 [†]
HCW ⁶ , kg	358.81	359.50	11.34	0.91
USDAYG ⁷	2.32	2.82	0.36	0.03 [†]
Ribeye Area, cm ²	82.81	79.78	2.94	0.16
Marbling Score	427.03	450.16	16.39	0.32

¹CON, control diet, ($n=30$) consisting of a typical ration for the Intermountain West, with 80% rolled barley, alfalfa, and corn silage;

²SB, sprouted barley diet ($n=30$) consisting of the same diet as control but corn silage and some rolled barley was replaced with sprouted barley at 20% dry matter inclusion;

³DMI: Dry matter intake;

⁴ADG: Average daily gain;

⁵G: F: Gain to feed ratio;

⁶HCW: Hot Carcass Weight;

⁷USDAYG: United States Department of Agriculture yield grade;

[†]Indicates a significant value.

Meat pH measurements

The pH of the meat was evaluated using previously described methods (Bendall, 1973). In brief, ~ 100 mg of tissue, powdered under liquid nitrogen, was homogenized at 1:10 (*w/v*) in ice-cold buffer (pH seven, 150 mM KCl, and five mM iodoacetic acid,) using a bead-beating homogenizer (TissueLyser LT, Qiagen, Hilden, Germany). Tissue homogenates were then centrifuged at 17,000 ×g for 10 min at room temperature and allowed to equilibrate to 25 °C for 10 additional min before being measured using an Orion Ross Ultra pH electrode coupled to an Orion Star A214 pH/ISE benchtop meter (ThermoFischer Scientific, Waltham, MA, USA). Before performing any pH readings, the pH electrode was calibrated at room temperature using Orion pH four, seven, and 10 buffer solutions (ThermoFischer Scientific, Pittsburgh, PA, USA).

Surface color and myoglobin form determination

Meat surface color was measured using a Konica Minolta chromameter (CR-400, Konica Minolta Sensing Inc., Osaka, Japan) with an eight mm aperture diameter, 2° observer angle, at illuminant D65, and myoglobin forms were determined spectrophotometrically using the Color Muse Spectro 1 (Variable, Inc., Chattanooga, TN, USA) with illuminant A, 10° observer angle, and eight mm aperture diameter parameters. Before each use, both instruments were calibrated according to their manufacturer guidelines and operated under manufacturer-recommended settings. Calibrations were performed prior to each time point but not between individual measurements. After wet aging for 12 d, a section of the loin was fabricated into two cm steaks and randomly allocated for either surface color and myoglobin form evaluation. Steaks designated for surface color and myoglobin form evaluation were allowed to bloom for 20 min then packaged in retail standard oxygen-permeable packaging and stored under refrigerated, lighted conditions to simulate a retail display case. At d three steaks were removed from the packaging, blotted dry, and allowed to bloom for 20 min at room temperature before measurements were taken. This process was repeated on d seven. This procedure allowed for consistent surface oxygenation prior to color and myoglobin analysis. Due to limitation in meat, only one steak per loin was allocated for color analysis. Three replicate scans were taken for surface color (King et al., 2023), and five scans were taken for the spectrophotometer (Wei et al., 2021) across different locations of each steak. Recorded surface color values were averaged and expressed in terms of Commission Internationale de l'Éclairage (CIE) *L** (lightness), *a** (redness), and *b** (yellowness). For spectrophotometric analysis, reflectance (*R*) values at 473, 525, 572, and 700 nm were used to calculate reflex attenuation (*A*) = log(1/*R*). Subsequently, percentages of myoglobin forms (MMb, DMb, and OMb) were then calculated using the following equations: %MMb = $\{1.395 - [(A_{572} - A_{700}) \div (A_{525} - A_{700})]\} \times 100$; %DMb = $\{2.375 \times [1 - (A_{473} - A_{700}) \div (A_{525} - A_{700})]\} \times 100$; %OMb = $\{100 - (\%MMb + \%DMb)\}$ (Krzywicki, 1979; King et al., 2023).

Drip loss

Drip loss was determined as described previously (Rasmussen and Andersson, 1996). Briefly, two cores approximately 10 g each were collected from each steak with a 2.5-cm-diameter hand-held coring device, weighed, and then placed individually into a drip loss tube (50 mL conical tube with steel wool to

elevate meat from bottom). Tubes were stored for 48 h at 4 °C. After which, the cores were removed from their respective tubes, blotted dry with paper towels, and weighed again. Drip loss was calculated as a percentage loss from the original weight of the sample.

WBSF and cook loss

Tenderness was evaluated using a WBSF (V-notch) attachment connected to TMS-Pro Texture Analyzer (Food Technology Co., Sterling, VA, USA), following the American Meat Science Association Sensory Guidelines (King et al., 2023). Briefly, steaks were weighed and cooked on separate electric clamshell-type grills to a consistent 71 °C internal temperature measured by digital thermocouple thermometers. Then, samples were equilibrated to room temperature, blotted dry, re-weighed, and stored overnight in a refrigerator (4 °C). Cook loss was calculated as a percentage of moisture lost from the initial weight of the steak. The next day, steaks were removed from the refrigerator, and six 1.27-cm-diameter cylindrical cores were removed from each steak parallel to the muscle fiber orientation using a handheld coring device. Each core was subsequently sheared perpendicular to the longitudinal orientation of the muscle fibers. Shear force was determined as the average maximum force of the six cores measured in Newtons (N).

Phytochemical analysis

Meat collected from the longissimus dorsi muscle after the 12 d wet aging period, and feed samples collected from the feed bunk, were pulverized in liquid nitrogen using an analytical mill (IKA® A 11 basic, Wilmington, NC, USA). Meat and feed samples, run in triplicate, were weighed out at 100 and 30 mg, respectively, and extracted with 1.25 ml of methyl tert-butyl ether: methanol (MTBE: MeOH) (2:1, *v*: *v*) spiked with internal standards (1 μM of 4-hydroxybenzoic acid ¹³C₆ and Apigenin D₅, and five μM of benzoic acid D₅ and trans-cinnamic acid D₇). Samples were homogenized at 30 oscillations/sec for two min twice using a Tissue Lyser II (Qiagen Retsch Tissue Lyser II, Germantown, MD, USA), sonicated in an ice-cold water bath for 15 minutes, and stored at -80 °C overnight to facilitate protein precipitation. The MTBE phase was removed by centrifugation at 16,000 ×g for 10 min at 4 °C after adding 750 μl of water. The organic phase was filtered (0.22 μm polytetrafluoroethylene (PTFE)) and stored at -80 °C until LC-MS/MS analysis. Protein pellets of feed samples were further treated with 2M NaOH (16 h at 30 °C) for bound phenolics extractions. pH was neutralized with formic acid and subjected to solid-phase extraction (Strata X-Pro, Phenomenex, Torrance, CA, USA) before liquid chromatograph tandem mass spectrometry (LC-MS/MS) analysis. Analysis was performed on SCIEX Hybrid Triple Quad 7500 mass spectrometer coupled to a Shimadzu UPLC system Nexera-LC-40. Separation was achieved using an ACQUITY UPLC HSS T3 1.8 μm 2.1x100 mm (Waters™) operated at 30 °C. The mobile phases consisted of 0.1% formic acid in water (A) and acetonitrile (B), and mobile phase flow rate was 0.6 ml/min. Gradient elution was 5% B from 0–2.1 mins, then linearly increased to 95% B over 10 min, and returned to 5% B at 14.5 mins. The system was equilibrated from 3.5 min in between injections. Five μL sample aliquots were injected in both positive and negative electrospray ionization modes, with ion spray voltages of -4000 V and +4500 V, respectively, with a source temperature

of 450 °C. Five μ L aliquots of samples were injected into the system. Double blanks (internal standard free extraction solvent) along with internal standards blank were run every 20 samples for quality control. Chromatographic data was processed using SCIEX OS 3.1 software (AB Sciex, Framingham, MA, USA). Peak integration was performed using MQ4 integration algorithm with signal/noise filter at three and noise percentage at 40%. The external standard curve used to quantify concentrations of compounds ranged from zero to 10 μ M injected as serial dilutions. Free and bound feed samples were summed to a total value for each sample. All phytochemical concentrations are expressed in μ g/100g.

Sensory analysis

A consumer acceptance panel was conducted utilizing untrained members of the Logan, Utah general population ($n=200$) at the USU sensory facility; the IRB #13656 approved all protocols and materials. Loins matched for WBSF, and marbling were cooked on separate clamshell grills until they reached an internal temperature of 71 °C as measured by digital thermocouples. The loins rested at room temperature for three min before being cut into similar 2.5 cm cubes. The samples were placed into aluminum cups, covered with segments of aluminum foil and a toothpick, then placed under a heat lamp for no more than 10 min to assure the sample's warmth. Cups were labelled with randomized 3-digit codes.

Panelists were provided with the two samples (presented balanced and randomized to each panelist) along with a napkin, unsalted saltines, and room-temperature water as a palate cleanser. Panelists utilized electronic software (SIMS 2000, Sensory Evaluation Software, Berkeley Heights, NJ, USA) to respond to their degree of liking of the following parameters: aroma, overall acceptance, flavor, tenderness, and juiciness. For all parameters, a balanced seven-point hedonic scale was used where one = dislike extremely, two = dislike very much, three = dislike moderately, four = neither like nor dislike, five = like moderately, six = like very much, and seven = like extremely. Participants then responded to the following demographic information: frequency of steak consumption, age, gender, ethnicity, education level, and income level.

Statistical analysis

Meat quality (Cooking loss, drip loss, pH, and WBSF) was analyzed using the PROC MIXED procedure of SAS (SAS v 9.4, Cary, NC, USA) to assess the main effect of diet. In the model, pen was used as a random effect and each individual animal as the experimental unit. All data are presented as the LS means $\pm$ SEM. Repeated measures were used to determine the main effects of diet, day, and diet $\times$ day for L^* , a^* , b^* , MMb, DMb, OMb. When diet differences were found to be significant ($P<0.05$), LS means were separated using Tukey-Kramer adjustments. When interactions were found to be significant, the main effects of that variable were not discussed individually.

Statistical analysis of the sensory attributes was performed in GraphPad Prism software (GraphPad Software, San Diego, CA) utilizing the paired, two-way T-test function for the mean acceptance score of each parameter $\pm$ SEM. Significance was declared at $P\leq 0.05$ and tendencies were discussed between $0.05 < P \leq 0.10$.

Statistical analysis of metabolomics data was performed using MetaboAnalyst 6.0, groups were compared by Welch's

t-test in the case of meat samples and ANOVA in the case of feed samples ($P<0.05$). Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) were utilized for visualization groups in feed and meat, respectively. A hierarchical, ranked clustering heatmap was generated to provide an intuitive visualization of the top 20 compounds between groups. For the heatmap, Pearson distance measure and Ward clustering were performed, and data were reported using the PLS-DA variable importance in projection (VIP) score. Random forest plots were developed to highlight the compounds in two separate groups.

Results and Discussion

This study evaluated whether including sprouted barley in the TMR of growing beef steers impacts meat quality. To the knowledge of the authors, no published research has specifically examined sprouted grains in this context, making these findings novel. As such, results of the present research are discussed in relation to established meat quality benchmarks and potential implications for beef production.

Water loss

Diet did not impact ($P\geq 0.23$) cook loss nor drip loss percentage (Table 3). During cooking, muscle fibers shrink, and therefore, so does their WHC. It has also been noted that the pH, sarcomere length, and salt content can be factors that affect cooking loss (Purslow et al., 2016). This results in a loss of weight as well as cooked meat yield. Cooking loss for both CON (33.5%) and SB (35.7%) were found to be similar to that of the industry standard (Macharáčková et al., 2021) within this range. Drip loss is defined as any fluid lost from a piece of meat when gravity is the only force acting on it (Fischer, 2007; Guo and Dalrymple, 2017). The amount of drip loss can be affected by many different things such as ultimate pH, storage temperature, and sarcomere length (Fischer, 2007). As previously stated, pH was within industry standards for ultimate pH, and storage temperature was a controlled factor. Research has shown that dietary factors such as increased vitamins, conjugated linoleic acid, and decreasing digestible carbohydrates can impact WHC in the meat that is produced (Cheng and Sun, 2008). However, in the present study it appears that these nutrients were not influenced to a degree where diet differences were observed.

Tenderness

There were no differences ($P=0.47$) between diet groups for WBSF (Table 3). It was found that CON and SB had a WBSF

Table 3. Meat quality measurements of the longissimus dorsi muscle from steers fed either a control diet (CON) or a diet containing sprouted barley (SB)

	CON	SB	SEM	P-Value
Meat pH	5.68	5.66	0.008	0.04
Cooking loss, %	19.87	21.55	0.97	0.23
Drip loss, %	1.38	1.23	0.14	0.45
WBSF, N	25.74	24.86	0.86	0.47

Meat pH, cooking loss %, drip loss %, and Warner-Bratzler Shear Force (WBSF) after a 12 d wet age period of the longissimus dorsi muscle from steers fed a control diet (CON; $n=30$) or a diet with a 20% inclusion of sprouted barley (SB; $n=30$).

of 25.74 N and 24.86 N, respectively, which would classify both as very tender (Miller et al., 1995; Destefanis et al., 2008; ASTM, 2011). It has been noted that tenderness can potentially vary between breed type, sex of the animal, the diet the animal received, as well as postmortem aging practices (Cuvelier et al., 2006; Cafferky et al., 2019; Yu et al., 2024). As aging time is extended, tenderness is increased due to cumulative proteolysis primarily by calpain, whose activity decreases over time (Koohmaraie and Geesink, 2006; Hughes et al., 2014; Yu et al., 2024). However, in the present study, aging time was controlled for and as previously stated, no difference due to diet was noted. Diet has been shown to directly impact the connective tissue formation and stability and growth rates before harvest can decrease myofibril fragmentation when meat is harvested (Aberle et al., 1981). Diets with increased energy content increase growth rates, resulting in rapid protein synthesis and greater portions of overall collagen in the muscle (Aberle et al., 1981). The resulting increase in collagen has fewer intermolecular crosslinks which result in highly soluble and less stable cross linked collagen fibers, which have been shown to increase meat tenderness (Goll et al., 1964; Hill, 1966). However, in the current study no differences were seen in tenderness due to diet.

Surface color

Meat color was assessed after fabrication on d zero, three, and seven after aging using a Konica Minolta colorimeter. No impacts of diet×day ($P \geq 0.21$) or diet ($P \geq 0.19$) were noted for

L^* , a^* , and b^* (Table 4). However, time ($P < 0.0001$) impacted for L^* , a^* , and b^* . The L^* increased ($P < 0.0001$) over the first three d then remained consistent ($P = 0.78$) until d seven (Table 4); a^* increased ($P < 0.0001$) from d zero to d three then decreased ($P < 0.0001$) at d seven (Table 4); b^* increased ($P < 0.0001$) at d three, then remained consistent ($P < 0.0001$) when measured at d seven (Table 4). A decrease in redness (a^*) is to be expected as the meat becomes more oxidized redness will decrease. Other studies found comparable results of decreasing a^* values over longer periods of time (Depenbusch et al., 2009; de Mello et al., 2018). The increase of a^* from d zero to three may reflect bloom development as the presence of oxygen impacts myoglobin forms. Additionally, factors such as pigment migration and light reflectance due to unblotted surface water can impact perceived redness (Ramanathan et al., 2010; Mahmood et al., 2017) resulting in inconsistent results. It has been shown that 80.5% of consumers view color as, “definitely important”, at the point of purchase (Testa et al., 2021; Benli and Yildiz, 2023). According to previously published research consumer acceptance of meat is when $L^* \geq 31.4$, $a^* \geq 16.4$, and $b^* \geq 6.5$ (Zhang et al., 2021). As seen in Table 4, all values for L^* , a^* , and b^* for both diet groups are above consumer acceptance thresholds, suggesting that SB in the diets of Angus cattle will not negatively impact meat color. It has been shown that steers finished on majority forages have yellow fat color and a darker color meat, whereas grain finished cattle tend to have white fat and a bright red meat color (Hughes et al., 2014; Pavan et al., 2023). As SB is neither a

Table 4. Meat color measurements of the longissimus dorsi muscle from steers fed either a control diet (CON) or a diet containing sprouted barley (SB)

	Time point			SEM	P-value		
	0	3	7		Diet	Day	Diet×Day
L^* ¹							
CON ²	43.31	45.06	45.17	0.54	0.25		0.91
SB ³	44.28	45.77	46.17				
All ⁴	43.79 ^a	45.41 ^b	45.67 ^b			<0.0001	
a^* ¹							
CON	21.85	23.36	20.85	0.14	0.19		0.21
SB	21.65	23.70	21.49				
All ²	21.75 ^a	23.53 ^b	21.17 ^c			<0.0001	
b^* ¹							
CON	11.89	13.78	12.68	0.09	0.27		0.41
SB	11.81	13.95	12.99				
All ²	11.85 ^a	13.87 ^b	12.83 ^c			<0.0001	
MMb ¹							
CON	6.52	17.61	23.75	0.62	0.05		0.68
SB	5.10	16.12	21.59				
All ²	5.80 ^a	16.87 ^b	22.67 ^c			<0.0001	
DMb ¹							
CON	5.34	4.68	8.34	0.27	0.18		0.53
SB	5.41	3.98	7.45				
All ²	5.38 ^a	4.33 ^b	7.89 ^c			<0.0001	
OMb ¹							
CON	88.13	77.71	67.91	0.88	0.08		0.57
SB	89.49	79.89	70.96				
All ²	88.81 ^a	78.81 ^b	69.44 ^c			<0.0001	

¹ L^* , a^* , b^* , deoxymyoglobin (DMb), oxymyoglobin (OMb), metmyoglobin (MMb) were assessed in boneless strip loins of steers fed a control diet or a diet with a 20% inclusion of sprouted barley (SB; $n = 30$);

²CON, control diet, ($n = 30$) consisting of a typical ration for the Intermountain West, with 80% rolled barley, alfalfa, and corn silage;

³SB, sprouted barley diet ($n = 30$) consisting of the same diet as control but corn silage and some rolled barley was replaced with sprouted barley at 20% dry matter inclusion;

⁴“All” is the value representing all samples measured to demonstrate the effects of time. Data are presented as a LS means $\pm$ SEM.

forage nor a concentrate, meat color and composition were unknown previously to the present study. Based on these results, SB content in the diet had no impact on color, suggesting that either the level of SB inclusion was not sufficient to affect color or that SB has no effect on color compared to the CON diet.

Myoglobin forms

Using a spectrophotometer, metmyoglobin (MMb), deoxymyoglobin (DMb), and oxymyoglobin (OMb) were measured. The MMb content was not significantly affected by the interaction of diet×day ($P=0.68$), but diet ($P=0.05$) as well as day ($P<0.0001$) did influence MMb content. No differences ($P\geq 0.29$) were seen between diets at each timepoint but an increase ($P<0.0001$) in MMb was detected over time for both diet groups (Table 4). This pattern aligns with the chemistry of myoglobin, the main heme protein that dictates meat color (Geletu et al., 2021). Depending on the oxidation state, it can exist as DMb, OMb, or MMb (Suman and Joseph, 2013). Discoloration is seen when OMb (ferrous, Fe^{2+}) oxidizes to MMb (ferric, Fe^{3+}), leading to a change from bright cherry-red to brown (Wallace et al., 1982), often accelerated under low O_2 conditions or aerobic microbial activity (Livingston and Brown, 1982). The increase in MMb can be detrimental to retailers as consumers have a preference for bright cherry red colored products and refrain from purchasing meat with noticeable amounts of MMb (Suman and Joseph, 2013; Testa et al., 2021). Increased MMb seen over time in the present study is consistent with expected oxidative changes in the meat.

The DMb content was also not affected by diet×day ($P=0.53$) or diet ($P=0.18$) but was affected by day ($P<0.0001$); DMb in both diet groups decreased ($P=0.05$) on day 3, then both increased ($P<0.0001$) on day seven (Table 4). Although absolute data differs, this is consistent previously published literature (Piao et al., 2022).

In addition, OMb had no diet×day ($P=0.57$) effect but did see a diet tendency ($P=0.08$) as well as a day effect ($P<0.0001$). This is explained by both CON and SB decreasing ($P<0.0001$) over the seven-day period (Table 4). This decline in OMb corresponds with the observed rise in MMb, which is detrimental for retailers. Consumers strongly prefer the bright cherry-red color associated with OMb and often avoid purchasing meat with noticeable MMb (Suman and Joseph, 2013; Testa et al., 2021). It has been estimated that 2.55% of all meat is discarded due to discoloration, representing a \$3.73 billion loss annually (Ramanathan et al., 2022).

As previously mentioned, diet can influence meat color; steers fed a forage-based diet typically produce darker meat

with yellow fat, while those fed a concentrate-based diet tend to have brighter red meat with white fat (Page et al., 2001; Hughes et al., 2014; Pavan et al., 2023). These color differences influence consumer preference, as color is typically the first characteristic evaluated at the time of purchase (Clinquart et al., 2022). In the present study, diet did influence some forms of myoglobin; however, these differences are negligible and could not be discerned by consumers or retailers (Greene et al., 1971). This would suggest that feeding SB would have no impact on myoglobin form or end-product color.

pH

In the current study, steaks produced from steers fed CON had an increased ($P=0.04$) pH compared to steaks from those fed SB (Table 3). Although differences were seen, both CON and SB both fall within normal pH range for beef of 5.5–5.7 (Dang et al., 2022). As the pH decreases to the isoelectric point (IP) of 5.1, water holding capacity (WHC) decreases (Lucarini et al., 2020). However, the rate and extent of pH decline can also play a part in WHC. A hastened pH decline can result in pale soft and exudative meat (PSE) commonly seen in pork, typically eliciting increased water loss. Consistent with this, despite the small difference in pH, no differences were observed in drip loss, cook loss, or color between diets (Tables 3 and 4). Previous work indicates that if pH decline stops prematurely, less water will be lost, and meat will likely be dark, firm, and dry (Hughes et al., 2014). Water-soluble pigment proteins, such as myoglobin, are the primary contributors to meat's unique red color. As such, there is a positive correlation between the increase in WHC and a dark red color because of the increase in myoglobin presence (Hughes et al., 2014). Together, these results indicate that while CON samples had a marginally increased ultimate pH, this difference was not biologically meaningful and is consistent with prior literature on acceptable postmortem pH ranges and their effects on beef quality. No known published research has specifically examined the effects of feeding SB to cattle and the subsequent impacts on the ultimate pH of meat.

Sensory

Due to response error, two panelists were dropped from the sensory panel ($n=198$). Panelists exhibited a wide range of demographic backgrounds, as summarized in Table 5. There were no differences ($P\geq 0.11$) between SB and CON in overall acceptance, aroma, flavor, tenderness, and juiciness, which indicates that SB diets may be used without considerable detriment to consumer acceptance (Table 6). The mean values for

Table 5. Top two demographic categories, their percentage of respondents, and the remaining percentage of respondents from other categories of the consumer acceptance panel for the frequency of steak consumption, age, gender, education level, ethnicity, and income

	Top category		Second category		Other responses
	Category	%	Category	%	%
Freq. of steak consumption	Once a month	28.6	Every two weeks and < once a month	Both 22.6	26.1
Age	18–24	24.6	75 or older	19.1	56.3
Gender	Man	49.7	Woman	47.7	2.5
Education Level	4-year college degree	34.2	Graduate degree	32.2	33.7
Ethnicity	White	83.9	Hispanic/Latino	6.5	9.5
Income	Less than \$30,000/year	28.6	\$60,000–89,999	19.6	51.8

all sensory parameters were valued between 4.19 to 4.66 for CON samples and 4.10 to 4.52 for SB samples (Table 6). Previous research has shown that finishing diet can alter consumer perception of flavor, particularly when comparing grass-fed and grain-fed systems, where differences in fatty acid profile and flavor compounds contribute to consumer preference (Van Elswyk and McNeill, 2014). In contrast, the absence of sensory differences in the present study suggests that replacing corn silage and some grain with sprouted barley does not produce detectable changes in sensory attributes.

Table 6. Consumer Sensory Analysis of steaks from the longissimus dorsi muscle from steers fed either a control diet (CON) or a diet containing sprouted barley (SB)

	CON ¹	SB ¹	SEM	P-Value
Aroma ²	4.19	4.10	0.07	0.31
Flavor ²	4.51	4.36	0.09	0.11
Tenderness ²	4.66	4.52	0.10	0.21
Juiciness ²	4.24	4.29	0.10	0.69
Overall Acceptance ²	4.40	4.31	0.09	0.23

¹CON, control diet, ($n=30$); SB, 20% inclusion of sprouted barley in the diet ($n=30$);

²Mean acceptance scores and SEM of the attributes measured with the consumer acceptance panel: aroma, overall acceptance, flavor, tenderness, and juiciness. A 7-point hedonic scale was used where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = neither like nor dislike, 5 = like moderately, 6 = like very much, and 7 = like extremely.

Phytochemicals in feed and meat

Phytochemicals are plant secondary metabolites that can have antioxidants, anti-inflammatory, and anti-microbial effects when consumed by cattle (Acamovic and Brooker, 2005; Poutaraud et al., 2017). Additionally, they may have the potential to impact flavor profile and nutritive quality of the beef that is produced from these animals (Provenza et al., 2019; Prache et al., 2020). In the present study, a comprehensive targeted phytochemical analysis identified 88 compounds across three distinct feed types: CON TMR, SB TMR, and the sprouted barley itself (SPB). Of the 88 identified compounds, 83 compounds varied significantly (ANOVA, FDR, $P < 0.05$) among the three different types of feed analyzed. Principal component (PC) analysis demonstrated complete separation of three types of feed samples where PC1 captured 75.4% of the variation and PC2 held 21.5% of total variation indicating metabolic difference among the three types of feed samples relative to phytochemical profiles (Figure 1). Notably, the CON TMR exhibited a unique metabolic profile compared to SPB with SB TMR being intermediate. Random Forest analysis identified metabolites significantly contributing to feed-type differentiation (Figure 2). The 3,4-dicaffeoylquinic acid (mean decrease accuracy > 0.013) was notably elevated in CON, whereas sinapic acid was elevated in SB TMR (mean decrease accuracy > 0.011). Conversely, luteolin (mean decrease accuracy > 0.01) was specifically enriched in the SPB samples, highlighting distinct metabolic signatures associated with each feed type.

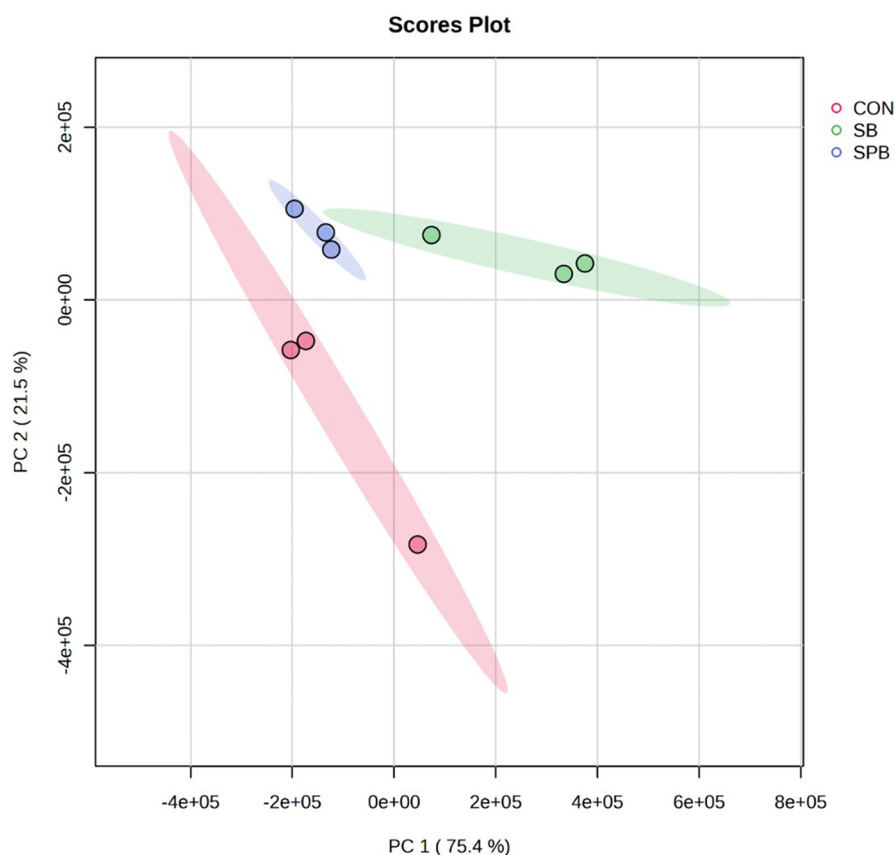


Figure 1. Scores plot showing feed metabolic separation between the control total mixed ration (CON) (red), sprouted barley total mixed ration (SB) (green), and barley sprout feed (SPB) (blue). Each feed type shows a distinct metabolic profile, but it is apparent that the CON TMR and SPB feed are more different from each other than the SB TMR, which is a combination of the control and barley sprout sample.

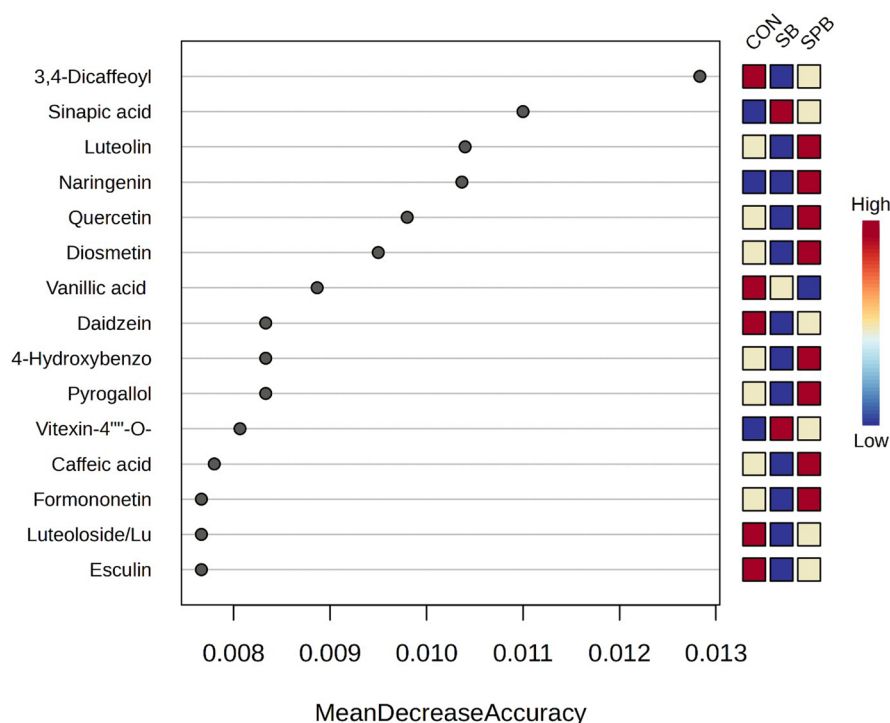


Figure 2. Random Forest plot of feed metabolites differentiating compounds between the control total mixed ration (CON), sprouted barley total mixed ration (SB), and barley sprout feed (SPB). Greater mean decreases in accuracy values indicate key metabolites, such as pyrogallol, trimethyl apigenin, and epicatechin and catechin, as significant metabolites that distinguish between samples. Red denotes high expression, and blue denotes low expression across group, while yellow denotes intermediate expression.

A heatmap analysis shows the CON TMR demonstrated elevated levels ($P < 0.05$) of 18 specific compounds, including p-coumaric acid, 3,4-dihydroxybenzoic acid, and vanillic acid 4- β -D-glucopyranoside (Figure 3). These phenolic acids are commonly associated with cereal grains and forage hays. The presence of coumestrol (phytoestrogen), a predominant component of alfalfa, indicates a significant proportion of alfalfa in the hay (Phillips et al., 1992). Furthermore, elevated levels of flavan-3-ols, such as epigallocatechin, and catechin, point towards the incorporation of legumes such as alfalfa and clover in CON TMR (Leoni et al., 2021).

Samples from the SB TMR were elevated ($P < 0.05$) for 23 compounds, notably caffeic acid, benzoic acid, pyrogallol, apigenin, and catechol (Figure 3). Caffeic and benzoic acids are prevalent polyphenols in fresh plants (Marchiosi et al., 2020), while apigenin and catechol are found in forage legumes such as red clover and alfalfa (Tucak et al., 2018). Several compounds, including 4-hydroxybenzoic acid, formononetin, chlorogenic acid, quercetin, secoisolariciresinol, and luteolin 7-O-glucoside, were common to both CON TMR and SPB feed, likely due to their ubiquitous presence in both conserved and fresh forages (Rapisarda and Abu-Ghannam, 2022).

A total of 12 phytochemicals were increased in the SPB feed compared to the CON TMR (all, $P < 0.05$), which were also significantly elevated in the SB TMR, indicating that the inclusion of sprouted barley can raise levels of various phytochemicals in finishing diets of livestock (Figure 3). Notably, saponarin, vitexin-4-O-glucoside, arbutin, and sinapic acid were elevated in SPB, intermediate in the SB TMT, and were lowest in the CON TMR. Trigonelline, a methylated niacin derivative abundant in cereals (Blake, 1954), was more prevalent in SPB feed than in the CON TMR, suggesting that

germination influences the breakdown of bound phenolic compounds (Dostalíková et al., 2024).

Despite considerable differences in feed samples, PC analysis revealed considerable metabolic overlap between the meat from steers fed SB or CON (Figure 4), indicating minimal metabolic alterations after a 20% DM inclusion of SPB into the diets of feeder cattle. Random Forest analysis identified metabolites significantly contributing to the differentiation of meat samples (Figure 5). Specifically, hippuric acid (mean decrease accuracy > 0.025) was notably elevated in CON meat, whereas 2-hydroxyisocaproic acid (mean decrease accuracy > 0.02) exhibited greater abundances in SB meat (Figure 5). Among the 23 detected metabolites in beef, only hippuric acid and 2-hydroxyisocaproic acid (HICA) demonstrated statistically significant differences (Welch's t-test, P -Value < 0.05). Hippuric acid is produced from the breakdown of dietary polyphenols by gut bacteria, then conjugated with glycine in the liver (Carrillo et al., 2016; De Simone et al., 2021). Hippuric acid is a major metabolite produced when the body processes dietary phenolic acids, especially from whole grains (Călinoiu & Vodnar, 2018). On the other hand, greater levels of HICA, a leucine metabolite, suggest a shift in amino acid metabolism, specifically increased leucine catabolism. This potentially reflect changes in protein or amino acid availability and utilization in the rumen or tissues.

Figure 6 shows phytochemicals distribution in all meat samples. Interestingly, only one control sample (ZC-27) had greater levels of stachydrine (also known as proline betaine) and ergothioneine. Ergothioneine is a sulfur-containing amino acid derived from histidine. It is produced by fungi and microbes in the soil and is commonly found in mushrooms (Beelman et al.,

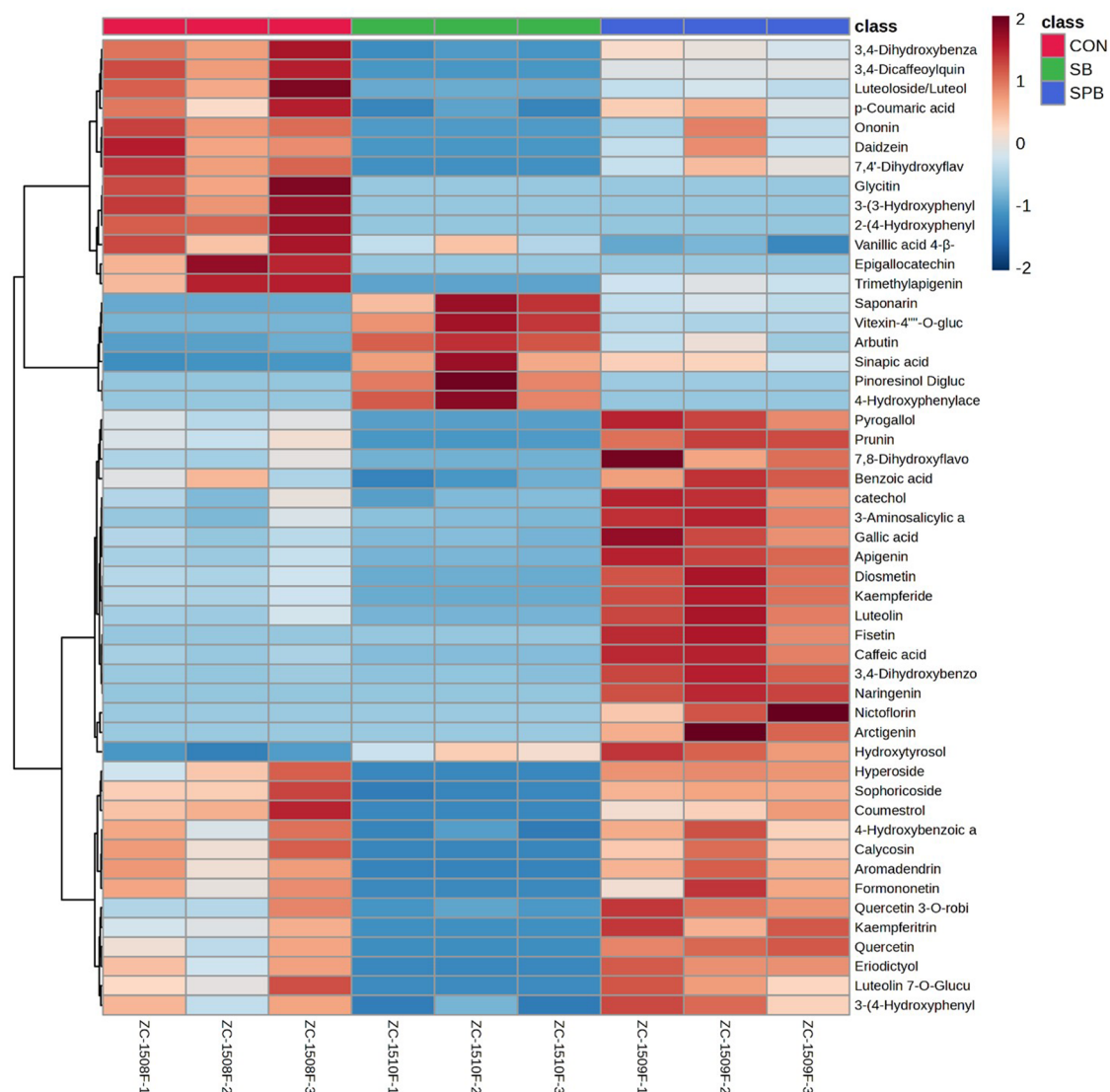


Figure 3. The heatmap shows distinct clustering of feed metabolites within samples and highlights the unique characteristics of specific feed types with red indicating high expression and blue indicating low expression under each respective feed type. Feed types include the control total mixed ration (CON) (red), sprouted barley total mixed ration (SB) (green), and barley sprout feed (SPB) (blue).

2021; Fujitani et al., 2018). On the other hand, only one SB sample (ZC-3) had greater abundance of L-kynurenine and cinnamoylglycine. L-kynurenine is a key metabolite in the tryptophan degradation pathway, often linked to immune modulation and oxidative stress (Tanaka et al., 2021), while cinnamoylglycine is a product of phenolic acid metabolism, potentially influenced by plant-based diets (Tanaka et al., 2021). In conclusion, the presence of elevated levels of specific metabolites in only individual samples suggests a heterogeneous distribution of compounds across the groups.

These findings suggest that although including sprouted barley in feeder cattle diets may alter specific metabolic pathways related to amino acid metabolism and phenolic compound processing due to differences in the diet's metabolomic profile, the overall impact on the beef metabolome is limited. This indicates that despite considerable phytochemical differences between SB and CON, the DM inclusion is likely too low to elicit a shift in the beef metabolome. While prior work has found that phytochemical metabolite abundance in grass-fed

and grain-fed beef differ by ~ 30–50%, an exclusive forage-based diet and a concentrate-based diet represents opposite ends of the spectrum (Evans et al., 2024; Krusinski et al., 2024). Additionally, various studies indicate that plant diversity is an important factor in altering the phytochemical richness of meat (van Vliet et al., 2021). Thus, future work should study if the inclusion of multiple sprouted products, at potentially greater inclusion rates of 20% DM, can impact the phytochemical richness of beef.

Conclusion

As beef producers face new challenges, sustainable and alternative production practices are needed to continue to meet the growing demand for beef production. New VFS are becoming more prevalent and have the potential to alleviate some of the common challenges drought and urbanization pose. However, beef producers' ability to market and sell their product is contingent on its quality, which can be influenced by the diets of

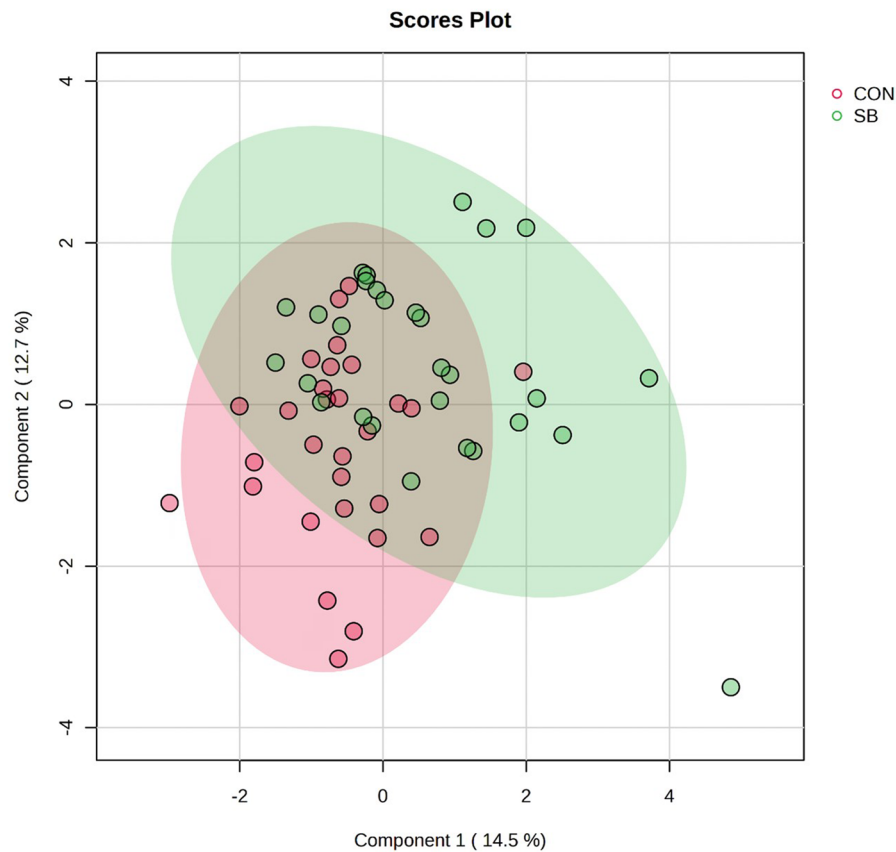


Figure 4. Scores plot showing meat metabolic separation between the control total mixed ration (CON; *n*=30) (red), sprouted barley total mixed ration (SB; *n*=30) (green). The homogeneity and overlap in the plot indicate only subtle differences were observed between groups.

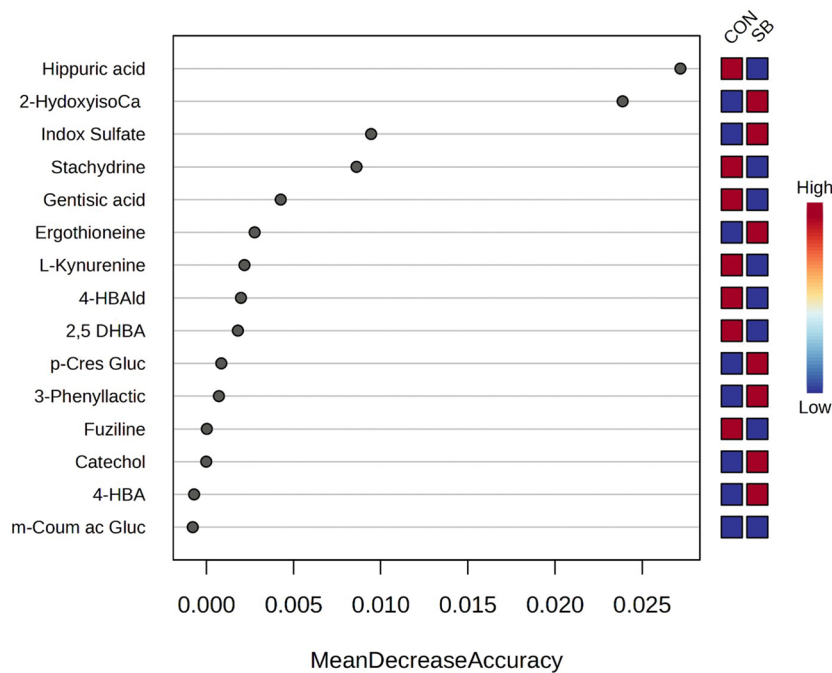


Figure 5. Random forest plot of meat metabolites differentiating cattle between the control total mixed ration (CON; *n*=30) (red) and sprouted barley total mixed ration (SB; *n*=30) (green). Greater mean decrease accuracy values indicate key metabolites, such as Hippuric Acid and 2-Hydroxyisocaproic Acid, as significant markers of the sprout diet's metabolic impact. Red denotes high expression, and blue denotes low expression across groups.

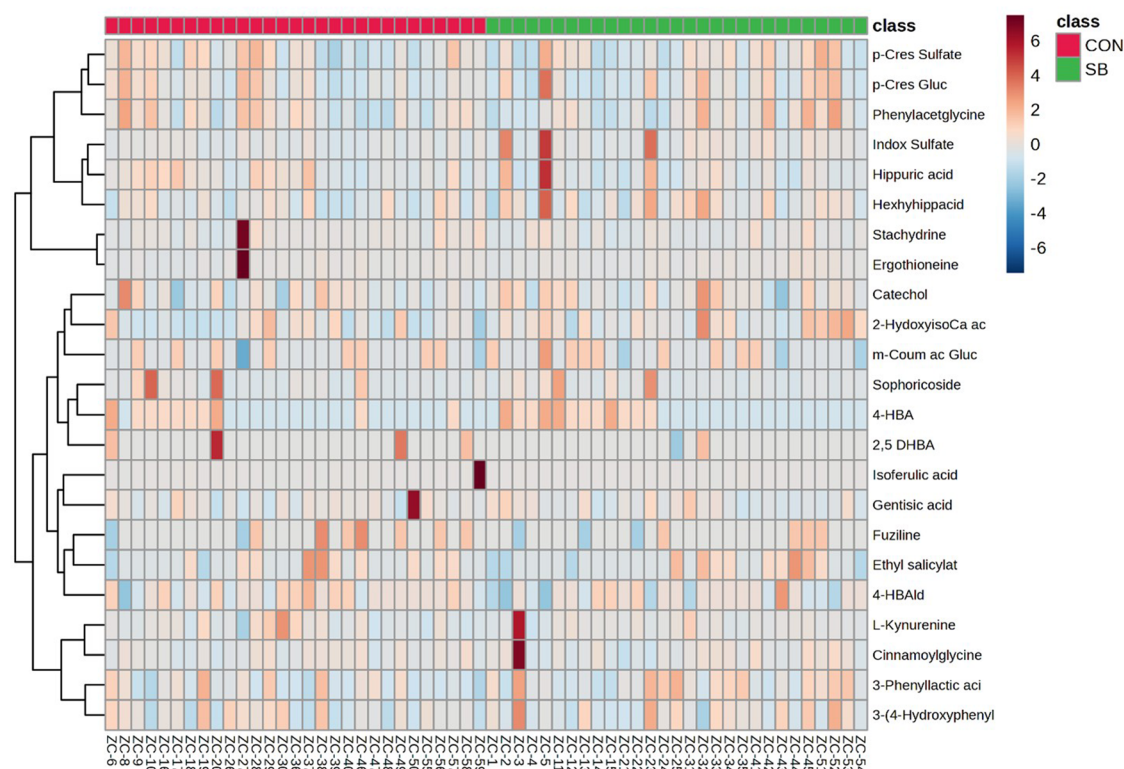


Figure 6. Heatmap of meat metabolite abundance in cattle fed the control total mixed ration (CON; $n=30$) (red) or sprouted barley total mixed ration (SB; $n=30$) (green), with red indicating high expression and blue indicating low expression. It shows no differences ($P>0.05$) in metabolic profiles between the barley sprout and control groups, as indicated by the lack of distinct clustering or pattern separation.

their cattle. Within the scope of this study, no significant differences were found between SB and CON diets pertaining to meat quality, metabolomic profiles, and sensory attributes. Further research, with studies utilizing larger samples sizes would need to be performed to validate results. Additionally, research will be needed to elucidate the economic feasibility of implementing VFS and SB diets in large-scale production operations.

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Author Contributions

Zachary Cal Crump (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Visualization, Writing—original draft, Writing—review & editing), Audrey Donna Lidgard (Formal analysis, Investigation, Writing—review & editing), Chandler D Stafford (Formal analysis, Methodology, Writing—review & editing), Taelie Ann Nauvoo Kennedy (Investigation, Writing—review & editing), Joseph Vinod Varre (Investigation, Writing—review & editing), Lamis Ali (Investigation, Writing—review & editing), Brady Morris Blackett (Investigation, Writing—review & editing), Robert Lynn Harding (Investigation,

Writing—review & editing), Sanjeewa Dilshan Ranathunga (Investigation, Writing—review & editing), Anthony Fernando Alberto (Investigation, Writing—review & editing), Bailee Lynn Brown (Investigation, Writing—review & editing), Bryce Wayne Roholt (Investigation, Writing—review & editing), Lillian Lelehua Okamoto (Investigation, Writing—review & editing), Muhammad Ahsin (Investigation, Writing—review & editing), Ryan Arthur Spurling (Investigation, Writing—review & editing), Stephan Van Vliet (Formal analysis, Investigation, Project administration, Writing—review & editing), Sulaiman Matarnah (Formal analysis, Investigation, Project administration, Writing—review & editing), and Kara J Thornton (Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing—review & editing)

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References

- Aberle, E. D., E. S. Reeves, M. D. Judge, R. E. Hunsley, and T. W. Perry. 1981. Palatability and muscle characteristics of cattle with controlled weight gain: time on a high energy diet. *J. Anim. Sci.* 52(4):757–763. <https://doi.org/10.2527/jas1981.524757x>
- Acamovic, T. and J. D. Brooker. 2005. Biochemistry of plant secondary metabolites and their effects in animals. *Proc. Nutr. Soc.* 64(3): 403–412. <https://doi.org/10.1079/PNS2005449>
- Ahamed, M. S., M. Sultan, R. R. Shamshiri, M. M. Rahman, M. Aleem, and S. K. Balasundram. 2023. Present status and challenges of fodder production in controlled environments: a review. *Smart Agric. Technol.* 3:100080. <https://doi.org/10.1016/j.atech.2022.100080>
- Alexandratos, N. and J. Bruinsma. 2012. World agriculture towards 2030/2050: the 2012 revision.
- ASTM. 2011. Standard specification for tenderness marketing claims associated with meat cuts derived from beef. West Conshohocken (PA): ASTM International.
- Beelman, R.B., J. P. Richie Jr, A. T. Phillips, M. D. Kalaras, D. Sun, and S. W. Duiker. 2021. Soil disturbance impact on crop ergothioneine content connects soil and human health. *Agronomy*. 11(11):2278.
- Bendall, J. R. 1973. 5 - POSTMORTEM CHANGES IN MUSCLE. In: G. H., Bourne, editor. *The structure and function of muscle*. 2nd ed. Academic Press. p. 243–309.
- Benli, H. and D. G. Yildiz. 2023. Consumer perception of marbling and beef quality during purchase and consumer preferences for degree of doneness. *Anim. Biosci.* 36(8):1274–1284. <https://doi.org/10.5713/ab.23.0003>
- Blake, C. O. 1954. Niacin metabolism in the corn seedling: the biosynthesis of trigonelline. *Am. J. Bot.* 41(3):231–238. [10.1002/j.1537-2197.1954.tb14331.x](https://doi.org/10.1002/j.1537-2197.1954.tb14331.x)
- Cafferky, J., R. M. Hamill, P. Allen, J. V. O'Doherty, A. Cromie, and T. Sweeney. 2019. Effect of breed and gender on meat quality of *M. longissimus thoracis et lumborum* muscle from crossbred beef bulls and steers. *Foods*. 8(5). <https://doi.org/10.3390/foods8050173>
- Calinoiu, L.F., and D. C. Vodnar. 2018. Whole grains and phenolic acids: A review on bioactivity, functionality, health benefits and bioavailability. *Nutrients*. 10(11):1615.
- Carrillo, J.A., Y. He, Y. Li, J. Liu, R. A. Erdman, T. S. Sonstegard, and J. Song. 2016. Integrated metabolomic and transcriptome analyses reveal finishing forage affects metabolic pathways related to beef quality and animal welfare. *Sci. Rep.* 6(1):25948.
- Cheng, Q. and D.-W. Sun. 2008. Factors affecting the water holding capacity of red meat products: a review of recent research advances. *Crit. Rev. Food Sci. Nutr.* 48(2):137–159. <https://doi.org/10.1080/10408390601177647>
- Clinquart, A., M. P. Ellies-Oury, J. F. Hocquette, L. Guillier, V. Santé-Lhoutellier, and S. Prache. 2022. Review: on-farm and processing factors affecting bovine carcass and meat quality. *Animal*. 16(Suppl 1):100426. <https://doi.org/10.1016/j.animal.2021.100426>
- Countryman, A. M., P. L. Paarlberg, and J. G. Lee. 2016. Dynamic effects of drought on the U.S. Beef supply chain. *Agric. Resour. Econom. Rev.* 45(3):459–484. <https://doi.org/10.1017/age.2016.4>
- Cuvelier, C., A. Clinquart, J. F. Hocquette, J. F. Cabaraux, I. Dufrasne, L. Istasse, and J. L. Hornick. 2006. Comparison of composition and quality traits of meat from young finishing bulls from Belgian blue, Limousin and Aberdeen angus breeds. *Meat Sci.* 74(3):522–531. <https://doi.org/10.1016/j.meatsci.2006.04.032>
- Dang, D. S., C. D. Stafford, M. J. Taylor, J. F. Buhler, K. J. Thornton, and S. K. Matarneh. 2022. Ultrasonication of beef improves calpain-1 autolysis and caspase-3 activity by elevating cytosolic calcium and inducing mitochondrial dysfunction. *Meat Sci.* 183:108646. <https://doi.org/10.1016/j.meatsci.2021.108646>
- de Mello, A. S., B. E. Jenschke, L. S. Senaratne, T. P. Carr, G. E. Erickson, and C. R. Calkins. 2018. Effects of finishing diets containing wet distillers grains plus solubles on beef quality attributes and fatty acid profile. *Meat Sci.* 136:16–22. <https://doi.org/10.1016/j.meatsci.2017.10.001>
- De Simone, G., C. Balducci, G. Forloni, R. Pastorelli, and L. Brunelli. 2021. Hippuric acid: Could become a barometer for frailty and geriatric syndromes?. *Ageing Res. Rev.* 72:101466.
- Depenbusch, B. E., C. M. Coleman, J. J. Higgins, and J. S. Drouillard. 2009. Effects of increasing levels of dried corn distillers grains with solubles on growth performance, carcass characteristics, and meat quality of yearling heifers1. *J. Anim. Sci.* 87(8):2653–2663. <https://doi.org/10.2527/jas.2008-1496>
- Destefanis, G., A. Brugiapaglia, M. T. Barge, and E. Dal Molin. 2008. Relationship between beef consumer tenderness perception and Warner–Bratzler shear force. *Meat Sci.* 78(3):153–156. <https://doi.org/10.1016/j.meatsci.2007.05.031>
- Dostaliková, L., P. Hlásná Čepková, D. Janovská, M. Jágr, P. Svoboda, V. Dvořáček, and I. Viehmannová. 2024. The impact of germination and thermal treatments on bioactive compounds of quinoa (*Chenopodium quinoa* willd.) seeds. *Eur. Food Res. Technol.* 250(5): 1457–1471. <https://doi.org/10.1007/s00217-024-04466-3>
- Duarte, T. L., B. Bolkenov, S. C. Klopatek, J. W. Oltjen, D. A. King, S. D. Shackelford, T. L. Wheeler, and X. Yang. 2022. Evaluating the shelf life and sensory properties of beef steaks from cattle raised on different grass feeding systems in the Western United States. *Foods*. 11(14) <https://doi.org/10.3390/foods11142141>
- Evans, N., J. Cloward, R. E. Ward, H. A. van Wietmarschen, N. van Eekeren, S. L. Kronberg, F. D. Provenza, and S. van Vliet. 2024. Pasture-finishing of cattle in Western U.S. rangelands improves markers of animal metabolic health and nutritional compounds in beef. *Sci. Rep.* 14(1):20240. <https://doi.org/10.1038/s41598-024-71073-3>
- Fischer, K. 2007. Drip loss in pork: influencing factors and relation to further meat quality traits. *J. Anim. Breed. Genet.* 124(s1):12–18. <https://doi.org/10.1111/j.1439-0388.2007.00682.x>
- Fujitani, Y., K. M. Alamgir, and A. Tani. 2018. Ergothioneine production using *Methylobacterium* species, yeast, and fungi. *J. Biosci. Bioeng.* 126(6):715–722.
- Geletu, U. S., M. A. Usmael, Y. Y. Mummed, and A. M. Ibrahim. 2021. Quality of cattle meat and its compositional constituents. *Vet. Med. Int.* 2021:7340495. <https://doi.org/10.1155/2021/7340495>
- Goll, D. E., W. G. Hoekstra, and R. W. Bray. 1964. Age-associated changes in bovine muscle connective tissue. I. Rate of hydrolysis by collagenase. *J. Food Sci.* 29(5):608–614. <https://doi.org/10.1111/j.1365-2621.1964.tb00418.x>
- Greene, B. E. H., Z. In-May, and W. Marel Ynn. 1971. Retardation of oxidative color changes in raw ground beef. *J. Food Sci.* 36(6):940–942. <https://doi.org/10.1111/j.1365-2621.1971.tb15564.x>
- Guo, B. and B. P. Dalrymple. 2017. Chapter 11 - Transcriptomics of meat quality. In: P. P., Purslow, editor. *New aspects of meat quality*. Cambridge: Woodhead Publishing. p. 259–320.
- Hill, F. 1966. The solubility of intramuscular collagen in meat animals of various ages. *J. Food Sci.* 31(2):161–166. <https://doi.org/10.1111/j.1365-2621.1966.tb00472.x>
- Hughes, J., S. Oiseth, P. Purslow, and R. Warner. 2014. A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. *Meat Sci.* 98(3):520–532.
- King, D. A., M. C. Hunt, S. Barbut, J. R. Claus, D. P. Cornforth, P. Joseph, Y. H. Kim, G. Lindahl, R. A. Mancini, M. N. Nair *et al.* 2023. American meat science association guidelines for meat color measurement. *Meat and Muscle Biol.* 6(4):104. <https://doi.org/10.22175/mmb.12473>
- Koohmaraie, M. and G. H. Geesink. 2006. Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. *Meat Sci.* 74(1):34–43. <https://doi.org/10.1016/j.meatsci.2006.04.025>

- Krusinski, L., I. C. F. Maciel, S. van Vliet, M. Ahsin, J. Adams, G. Lu, C. A. Bitler, J. E. Rowntree, and J. I. Fenton. 2024. Fatty acids and secondary metabolites can predict grass-finished beef and supplemental cattle feeds. *NPJ Sci. Food*. 8(1):73. <https://doi.org/10.1038/s41538-024-00315-5>
- Krzywicki, K. 1979. Assessment of relative content of myoglobin, oxymyoglobin and metmyoglobin at the surface of beef. *Meat Sci.* 3(1):1–10.
- Leeper, R. D., R. Bilotta, B. Petersen, C. J. Stiles, R. Heim, B. Fuchs, O. P. Prat, M. Palecki, and S. Ansari. 2022. Characterizing U.S. drought over the past 20 years using the U.S. drought monitor. *Int. J. Climatol.* 42(12):6616–6630. <https://doi.org/10.1002/joc.7653>
- Leoni, F., H. Hazrati, I. S. Fomsgaard, A.-C. Moonen, and P. Kudsk. 2021. Determination of the effect of Co-cultivation on the production and root exudation of flavonoids in four legume species using LC–MS/MS analysis. *J. Agric. Food Chem.* 69(32):9208–9219. <https://doi.org/10.1021/acs.jafc.1c02821>
- Livingston, D. J., and W. Brown. 1982. The chemistry of myoglobin and its reactions. *Food Technology*. 35(5):244–252.
- Lucarini, M., A. Durazzo, F. Sciubba, M. E. Di Cocco, R. Gianferri, M. Alise, A. Santini, M. Delfini, and G. Lombardi-Boccia. 2020. Stability of the meat protein type I collagen: Influence of pH, ionic strength, and phenolic antioxidant. *Foods*. 9(4) <https://doi.org/10.3390/foods9040480>
- Macharáčková, B., K. Bogdanovičová, F. Ježek, J. Bednář, D. Haruštiaková, and J. Kameník. 2021. Cooking loss in retail beef cuts: the effect of muscle type, sex, ageing, pH, salt and cooking method. *Meat Sci.* 171:108270. <https://doi.org/10.1016/j.meatsci.2020.108270>
- Mahmood, S., B. C. Roy, I. L. Larsen, J. L. Aalhus, W. T. Dixon, and H. L. Bruce. 2017. Understanding the quality of typical and atypical dark cutting beef from heifers and steers. *Meat Sci.* 133:75–85. <https://doi.org/10.1016/j.meatsci.2017.06.010>
- Marchiosi, R., W. D. dos Santos, R. P. Constantin, R. B. de Lima, A. R. Soares, A. Finger-Teixeira, T. R. Mota, D. M. de Oliveira, M. d P. Foletto-Felipe, J. Abrahão *et al.* 2020. Biosynthesis and metabolic actions of simple phenolic acids in plants. *Phytochem. Rev.* 19(4):865–906. <https://doi.org/10.1007/s11101-020-09689-2>
- Miller, M., L. Hoover, K. Cook, A. Guerra, K. Huffman, K. Tinney, C. Ramsey, H. Brittin, and L. Huffman. 1995. Consumer acceptability of beef steak tenderness in the home and restaurant. *J. Food Sci.* 60(5):963–965.
- Morales Gómez, J. F., D. S. Antonelo, M. Beline, B. Pavan, D. B. Bambil, P. Fantinato-Neto, A. Saran-Netto, P. R. Leme, R. S. Goulart, D. E. Gerrard *et al.* 2022. Feeding strategies impact animal growth and beef color and tenderness. *Meat Sci.* 183:108599. <https://doi.org/10.1016/j.meatsci.2021.108599>
- Nogoy, K. M. C., B. Sun, S. Shin, Y. Lee, X. Zi Li, S. H. Choi, and S. Park. 2022. Fatty acid composition of grain- and grass-fed beef and their nutritional value and health implication. *Food Sci. Anim. Resour.* 42(1):18–33. <https://doi.org/10.5851/kosfa.2021.e73>
- Page, J., D. Wulf, and T. Schwotzer. 2001. A survey of beef muscle color and pH. *J. Anim. Sci.* 79(3):678–687.
- Pavan, E., L. B. Pouzo, M. L. Testa, C. S. Maglietti, J. P. Mazzucco, and A. M. Pardo. 2023. Impact of the duration of rearing and finishing periods on steer performance and carcass and meat quality characteristics. *Livest. Sci.* 276:105313. <https://doi.org/10.1016/j.livsci.2023.105313>
- Phillips, D. A., C. M. Joseph, and C. A. Maxwell. 1992. Trigonelline and stachydrine released from alfalfa seeds activate NodD2 protein in *Rhizobium meliloti*. *Plant Physiol.* 99(4):1526–1531. <https://doi.org/10.1104/pp.99.4.1526>
- Piao, S., S. Li, J. Gonzalez, J. Hodgen, T. A. Houser, E. A. E. Boyle, and T. G. O'Quinn. 2022. Daily quantification of myoglobin forms on beef longissimus lumborum steaks over 7 days of display. *Meat Muscle Biol.* 6(1):12562. <https://doi.org/10.7275/12562>
- Poutaraud, A., A. Michelot-Antalik, and S. Plantureux. 2017. Grasslands: a source of secondary metabolites for livestock health. *J. Agric. Food Chem.* 65(31):6535–6553. <https://doi.org/10.1021/acs.jafc.7b00425>
- Prache, S., B. Martin, and M. Coppa. 2020. Review: Authentication of grass-fed meat and dairy products from cattle and sheep. *Animal.* 14(4):854–863. <https://doi.org/10.1017/S1751731119002568>
- Provenza, F. D., S. L. Kronberg, and P. Gregorini. 2019. Is grassfed meat and dairy better for human and environmental health? *Front. Nutr.* 6:26. <https://doi.org/10.3389/fnut.2019.00026>
- Purslow, P. P., S. Oiseth, J. Hughes, and R. D. Warner. 2016. The structural basis of cooking loss in beef: variations with temperature and ageing. *Food Res. Int.* 89(Pt 1):739–748. <https://doi.org/10.1016/j.foodres.2016.09.010>
- Ramanathan, R., L. H. Lambert, M. N. Nair, B. Morgan, R. Feuz, G. Mafi, and M. Pfeiffer. 2022. Economic loss, amount of beef discarded, natural resources wastage, and environmental impact due to beef discoloration. *Meat Muscle Biol.* 6(1):13218–13218. <https://doi.org/10.22175/mmb.13218>
- Ramanathan, R., R. Mancini, B. Naveena, and M. Konda. 2010. Effects of lactate-enhancement on surface reflectance and absorbance properties of beef longissimus steaks. *Meat Sci.* 84(1):219–226.
- Rapisarda, S., and N. Abu-Ghannam. 2022. Polyphenol characterization and antioxidant capacity of Multi-Species swards grown in Ireland—environmental sustainability and nutraceutical potential. *Sustainability*. 15(1):634.
- Rasmussen, A., and M. Andersson. 1996. New method for determination of drip loss in pork muscles. In: *Proceedings of the 42nd international congress of meat science and technology*, Lillehammer, Norway. P. 286287.
- Suman, S. P., and P. Joseph. 2013. Myoglobin chemistry and meat color. *Annu. Rev. Food Sci. Technol.* 4(1):79–99. <https://doi.org/10.1146/annurev-food-030212-182623>
- Tarnonsky, F., K. Hochmuth, A. DiCostanzo, and N. DiLorenzo. 2023. Effects of replacing corn silage with alfalfa haylage in growing beef cattle diets on performance during the growing and finishing period. *J. Anim. Sci.* 101. <https://doi.org/10.1093/jas/skac397>
- Tanaka, M., and L. Vécsei. 2021. Monitoring the kynurenine system: Concentrations, ratios or what else?. *Adv. Clin. Exp. Med.* 30(8):775–778.
- Testa, M. L., G. Grigioni, B. Panea, and E. Pavan. 2021. Color and marbling as predictors of meat quality perception of Argentinian consumers. *Foods*. 10(7):1465.
- Tucak, M., D. Horvat, T. Cupic, G. Krizmanic, V. Tomas, M. Ravlic, and S. Popovic. 2018. Forage legumes as sources of bioactive phytoestrogens for use in pharmaceuticals: a review. *Curr. Pharm. Biotechnol.* 19(7): 537–544. <https://doi.org/10.2174/1389201019666180730165917>
- U.S. Department of Agriculture. 2023. *Farms and land in farms 2022 summary*. Washington: U.S. Department of Agriculture.
- Van Elswyk, M. E., and S. H. McNeill. 2014. Impact of grass/forage feeding versus grain finishing on beef nutrients and sensory quality: the U.S. experience. *Meat Sci.* 96(1):535–540. <https://doi.org/10.1016/j.meatsci.2013.08.010>
- van Vliet, S., F. D. Provenza, and S. L. Kronberg. 2021. Health-Promoting phytonutrients are higher in grass-fed meat and milk. *Front. Sustain. Food Syst.* 4
- Vasta, V., and G. Luciano. 2011. The effects of dietary consumption of plants secondary compounds on small ruminants' products quality. *Small Rumin. Res.* 101(1-3):150–159. <https://doi.org/10.1016/j.smallrumres.2011.09.035>
- Wallace, W. J., R. A. Houtchens, J. C. Maxwell, and W. S. Caughey. 1982. Mechanism of autooxidation for hemoglobins and myoglobins. Promotion of superoxide production by protons and anions. *J. Biol. Chem.* 257(9):4966–4977. [https://doi.org/10.1016/S0021-9258\(18\)34620-9](https://doi.org/10.1016/S0021-9258(18)34620-9)
- Wei, X., S. Lam, B. M. Bohrer, B. Uttaro, O. López-Campos, N. Prieto, I. L. Larsen, and M. Juárez. 2021. A comparison of fresh pork colour measurements by using four commercial handheld devices. *Foods*. 10(11):2515.
- Yu, Q., X. Gu, Q. Liu, R. Wen, and C. Sun. 2024. Effect of wet-aging on meat quality and exudate metabolome changes in different beef muscles. *Food Res. Int.* 184:114260. <https://doi.org/10.1016/j.foodres.2024.114260>
- Zhang, Y., B. W. Holman, Y. Mao, X. Chen, X. Luo, D. L. Hopkins, and Y. Zhang. 2021. Determination of a pH threshold for dark cutting beef based on visual evaluation by Asian consumers. *Meat Sci.* 172:108347.