



## Flavor Characterization of Grass- and Grain-Fed Australian Beef *Longissimus Thoracis* Aged 35 to 65 Days Postmortem

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**Abstract:** Our objective was to investigate the effects of extended aging on the flavor characteristics of Australian grass- and grain-fed beef *longissimus thoracis*. Ribeye rolls from Australian grass- and grain-fed beef carcasses were collected, randomly assigned to one of 4 postmortem aging days (35, 45, 55, and 65), vacuum packaged, and shipped under refrigeration (0°C–2°C) to Texas Tech University (Lubbock, TX). Aged *longissimus thoracis* were fabricated on their respective aging day into 2.54-cm steaks, vacuum packaged individually, and frozen (–24°C) until further analyses. According to trained flavor panelists, beef flavor identity, fat-like, metallic, umami, bitter, and sweet flavors were not influenced by diet or postmortem aging ( $P > 0.05$ ). Diet influenced ( $P < 0.05$ ) liver-like, rancid, grassy, and sour flavors, as well as juiciness. For all flavors except liver-like, grass-fed samples had stronger flavors than grain-fed samples. Postmortem aging influenced ( $P < 0.05$ ) bloody/serummy, liver-like, rancid, and grassy flavors, along with tenderness and juiciness. Flavor detection typically became stronger for those flavors as postmortem aging increased; however, bloody/serummy, juiciness, and tenderness generally did not follow a linear trend as postmortem aging increased. Content of acetic acid and hexanal were each greater ( $P < 0.05$ ) in grain-fed beef. The majority of lipid oxidation compounds were most prominent ( $P < 0.05$ ) in samples aged 45 and 55 d, while content in 35 and 65 d were lower and did not differ ( $P > 0.05$ ). Aging also influenced content of acetic acid and ethanol ( $P \leq 0.04$ ), which increased with aging duration. For 1-octen-3-ol, grain-fed samples aged 65 d had the greatest ( $P < 0.05$ ) content compared with all other diet and aging combinations. Results indicate that aging up to 65 d had no impact on beef flavor identity and umami, but it led to stronger generation of certain off-flavors such as rancid, grassy, and liver-like.

**Key words:** beef, descriptive sensory attributes, diet, flavor, postmortem aging, volatile compounds

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## Introduction

Australia is a substantial exporter of chilled beef around the world, and the United States is the third largest recipient (in volume) of Australian chilled beef, importing 140.9 million lb in 2018 (Department of Agriculture, Water and the Environment, 2019). Australia relies evenly on grass and grain finishing cattle, especially considering cattle presented for Meat Standards Australia carcass grading (MLA, 2019). Finishing cattle on grain versus grass can influence fatty acid profiles, volatile compounds, and

ultimately flavor of the meat produced from those animals (Elmore et al., 2004; Maughan et al., 2012; Fruet et al., 2018). Moreover, transit time for Australian exported goods could be anywhere from 25 to 40 days from the time it leaves Australia until reaching its destination in the US (Australian Trade and Shipping, 2019); however, this does not include distribution time. Therefore, by the time chilled Australian beef reaches a US consumer, the product could easily be 45 days post-slaughter. However, little research has been conducted characterizing beef flavor at extended postmortem aging periods while also considering the cattle finishing system.

Shelf life refers to the deterioration in organoleptic and sensory characteristics, such as color, odor, and taste, that occur during meat storage (MLA, 2016). Meat may become unsuitable for use or consumption when a certain microbiological limit is breached or unacceptable changes occur in color, odor, or taste (MLA, 2016). The end of a product's shelf life may be difficult to define precisely as acceptability changes from consumer to consumer. Until recently, 100 d was the commonly accepted shelf life of Australian vacuum-packaged beef stored at  $-1^{\circ}\text{C}$  during shipping and storage (Small et al., 2012), but more conservative limits (77–84 d) are in place for some Australian exports to account for potential deficiencies in the supply chain (MLA, 2016). Recent studies have suggested that a shelf life between 20 and 30 wk (140–210 d) for vacuum-packaged beef can be achieved if temperature is well controlled and maintained from  $-2^{\circ}\text{C}$  to  $-1^{\circ}\text{C}$  (Small et al., 2012; Hughes et al., 2015).

Although postmortem aging has a well-established positive influence on meat tenderness (Gruber et al., 2006; Brewer and Novakofski, 2008; Juárez et al., 2010; Hughes et al., 2015), its impacts on beef flavor are inconsistent and less well defined. In one instance, consumers liked the flavor of beef *longissimus lumborum* aged for 12 wk more than the flavor of beef aged for 2 wk, but no further improvement to flavor liking scores was observed from aging beef for 20 wk (Hughes et al., 2015). According to Brewer and Novakofski (2008), postmortem aging up to 21 d had no influence on beef flavor; however, extended postmortem aging can promote the development of undesirable flavor characteristics and the reduction of beef flavor intensity (Juárez et al., 2010; Lepper-Blilie et al., 2016). Even in samples that were only aged up to 14 d postmortem, desirable flavors such as beefy, brothy, brown-caramel, and sweet declined, while less favorable flavors such as bitter and sour increased (Spanier et al., 1997).

According to Wang et al. (2013), extended aging releases free fatty acids, which react with protein and other flavor precursors, thus affecting the aroma and flavor of aged meat. Aging impacts various volatile compounds in beef muscles and often results in decreased desired flavor compounds while undesired flavor compounds increase with aging from 7 to 14 days (Stetzer et al., 2008). Flavor is a very important quality attribute in beef during postmortem aging, during which proteolytic degradation of muscle fibers into short peptides, nucleotides, free amino acids, and other nitrogen-containing compounds contributes to meat flavor enhancement. Additionally, chemical and biochemical

reactions, particularly lipid oxidation and protein oxidation, could cause flavor deterioration due to the generation of secondary lipid oxidation products and sulfur compounds on the exposed surfaces of carcasses and meat cuts (Martinaud et al., 1997; Spanier et al., 1997).

Shelf life can be extended to 20+ wk for Australian chilled beef, but off-odors and off-flavors can still be detected (Rodas-González et al., 2011; Small et al., 2012). Moreover, postmortem aging can elicit the development of undesirable flavor characteristics. Given these findings, there is a need to characterize the flavor of Australian vacuum-packaged beef that has been exposed to extended periods of postmortem aging, especially in the export market where the product is destined to be consumed. Therefore, this study was conducted to investigate the effects of extended aging up to 65 d postmortem on the subjective and objective flavor characteristics of Australian grass- and grain-fed beef *longissimus thoracis*.

## Materials and Methods

### Carcass selection

Animals were randomly selected ( $n = 96$ ; 48 per diet treatment) at a commercial abattoir near Brisbane, Australia, to equally represent grain- and grass-fed diets. Live animal information pertaining to diet (grass vs. grain) was made available through the animal identification system in Australia, but no data were collected on farm. Sixteen animals were harvested per day over a period of 6 d. On the first day, grain-fed animals were slaughtered, and then grass-fed were slaughtered the following day. This pattern continued through 6 slaughter days. Carcasses were fabricated at 24 h postmortem, and ribeye rolls (Institutional Meat Purchase Specifications #112A) were collected from one side and randomly assigned to 1 of 4 aging days (35, 45, 55, or 65 d postmortem). The ribeye rolls were vacuum packaged, stored at  $0^{\circ}\text{C}$  to  $2^{\circ}\text{C}$ , and transported to Texas Tech University (Lubbock, TX) via refrigerated air freight and road transport. Samples arrived prior to 35 d postmortem and were held at refrigerated temperatures ( $2^{\circ}\text{C}$  to  $4^{\circ}\text{C}$ ) until steak fabrication.

### Subprimal fabrication and designation

At the completion of each designated aging time, subprimals were cut into 2.5-cm steaks and labelled accordingly from the posterior to anterior end. The first

(most posterior) steak of each ribeye roll was utilized in determining composition and pH, the second steak was assigned to trained flavor panel analysis, the third to cooked volatiles analysis, and the fourth steak was assigned to Warner-Bratzler shear force (WBSF) evaluation. The second steak from each subprimal was allowed to oxygenate at 2°C to 4°C for approximately 20 min before objective color was obtained using a handheld colorimeter (Minolta CR-400 Chroma Meter; Konica Minolta Sensing Americas, Ramsey, NJ) with illuminant A, a standard observer angle of 10°, and a 1-cm-diameter aperture. Three independent readings were taken to obtain  $L^*$  (lightness),  $a^*$  (redness), and  $b^*$  (yellowness) values, which were averaged before statistical analyses. After cutting, steaks were appropriately labelled according to position and designated analyses, vacuum packaged, and frozen (−24°C) until further analyses.

### Proximate analysis

Proximate analysis was performed using only the *longissimus thoracis* muscle to determine the percentage of fat, moisture, and protein. Frozen steaks were thawed at 2°C to 4°C for 24 h prior to analysis. All subcutaneous fat, intermuscular fat, and heavy connective tissues were removed from each sample. Samples were cubed and ground 3 times through a 4-mm plate table top grinder (Cabela's deluxe meat grinder, Model #: 54-1091). Samples were analyzed using an AOAC International official method (Anderson, 2007) using a near infrared spectrophotometer (FoodScan, FOSS NIRsystems, Inc., Laurel, MD). A petri dish disc was filled with approximately 100 g of sample, leveled with a plastic spatula, and placed in the FOSS FoodScan machine to obtain results.

### pH determination

Ten grams of ground sample was retained after proximate analysis and placed into a 150-mL beaker. Ninety milliliters of distilled water was added to that beaker. The mixture was agitated for 30 s at 300 revolutions per minute with a magnetic stirrer (Thermo Scientific Cimarec Stirring Hot Plate, 7" × 7" Ceramic; 120 VAC, China). After homogenization, filter paper (#415 VWR North American, Category #: 28320-100) was shaped into a cone and placed in the 150-mL beaker to allow the water to diffuse to the center of the cone. An electrode connected to a pH meter (Oakton MS PH01; Vernon Hills, IL) was then placed in the center of the cone to measure the pH of the dilution, and the results were recorded.

The pH of each sample was determined as the average of 3 ground samples.

### Cooked product preparation

For evaluations requiring cooking (trained sensory panels, WBSF, and volatile flavor compound analysis), steaks were prepared following a common cooking protocol outlined in the American Meat Science Association sensory guidelines (AMSA, 2015). Frozen steaks were thawed at 2°C to 4°C for 24 h prior to cooking and were trimmed of subcutaneous and intermuscular fat. Three steaks were cooked at one time using an electric clamshell grill (Cuisinart Griddler Deluxe, Cuisinart, East Windsor, NJ) to a medium degree of doneness (71°C endpoint temperature) monitored using a digital thermometer (Thermopen, Classic SuperFast, Thermoworks, American Fork, UT). Peak temperature was recorded after removal from the grill.

### Trained sensory panels

Descriptive flavor panelists were trained according to the American Meat Science Association sensory guidelines (AMSA, 2015). Eight panelists were selected to participate in each panel from a pool of 15 trained panelists composed mostly of graduate students from the Department of Animal and Food Sciences at Texas Tech University (Lubbock, TX). Each panelist completed more than 20 h of descriptive sensory training. Table 1 shows the flavor attributes, references, and scores used for training panelists.

Steaks were cooked as described previously. After cooking, steaks were sliced into cubes measuring 1.3 cm × 1.3 cm × cooked steak thickness and served in 60-mL plastic cups, identified with randomly generated 4-digit unique identification codes, and placed into a preheated chamber maintained at approximately 55°C (Cambro Ultra Heated Holding Pan Carrier, 214UPCH400, Webstaurant Store, Lititz, PA) to keep them warm before evaluation. Samples were stored for about 10 min before serving. One sample was served at a time, and panelists evaluated 8 samples during a 30-min session. Panelists used unsalted saltine crackers and filtered water to cleanse their palates between samples. Evaluations were performed under red light to eliminate any bias from appearance. Panelists evaluated 12 flavor descriptors (beef flavor identity, bloody/serumy, fat-like, liver-like, umami, sweet, salty, bitter, sour, grassy, metallic, and rancid), juiciness, and tenderness on 100-mm line scales (0 = slight; 50 = moderate; and 100 = strong). Data were collected on iPad (5th generation; Model A1822 EMC 3017; Apple,

**Table 1.** Definitions and references used for beef flavor attributes, as well as overall juiciness and tenderness, as adapted from the AMSA sensory guidelines (2015)

| Sensory Attribute           | Definition   | Reference (Score)  |
|-----------------------------|--|--|
| <b>Beef Flavor Identity</b> | The amount of beef identity in a sample  | Swanson's beef broth diluted with water 50:50 (30)<br>80% lean ground chuck—pan fried until brown (50)<br>Beef brisket (75)      |
| <b>Bloody/Serumy</b>        | An aromatic associated with blood in cooked meat. Closely related to metallic.   | USDA Choice strip steak cooked to 60°C (40)  |
| <b>Fat-like</b>             | Aromatic associated with cooked animal fat   | Beef brisket cooked to 71°C (10)<br>Ground beef 90/10—pan fried until brown (30)<br>Ground beef 70/30—pan fried until brown (60) |
| <b>Liver-like</b>           | Aromatics associated with cooked organ meat/liver  | Flat iron steak cooked to 71°C (20)  |
| <b>Sour</b>                 | Fundamental taste factor associated with a citric acid solution  | 0.015% citric acid solution (10)<br>0.050% citric acid solution (25)   |
| <b>Bitter</b>               | The fundamental taste associated with caffeine solution  | 0.01% caffeine solution (15)<br>0.02% caffeine solution (25)   |
| <b>Sweet</b>                | A fundamental taste associated with sucrose  | 0.50% sucrose solution (25)  |
| <b>Metallic</b>             | Impression of slightly oxidized metal  | Dole canned pineapple juice (40)   |
| <b>Rancid</b>               | Aromatics commonly associated with oxidized fat and oils; may include cardboard, painty, varnish, and fishy                    | Microwaved vegetable oil—3 min (45)<br>Microwaved vegetable oil—5 min (55)   |
| <b>Umami</b>                | Flat, salty, and somewhat brothy with taste similar to glutamate, salts of amino acids, and other molecules called nucleotides | Swason's 99% fat- and sodium-free beef broth (30)  |
| <b>Grassy</b>               | Brown/green dusty aromatics associated with dry grasses, hay, dry parsley, and tea leaves                                      | Dry parsley (40)   |
| <b>Juiciness</b>            |  | Strip steak cooked to 85°C (20)<br>Strip steak cooked to 71°C (50)<br>Strip steak cooked to 60°C (75)                            |
| <b>Tenderness</b>           |  | Eye of round steak cooked to 85°C (20)<br>Strip steak cooked to 71°C (55)<br>Tenderloin steak cooked to 65°C (90)                |

AMSA = American Meat Science Association; USDA = US Department of Agriculture.

Cupertino, CA) electronic devices preloaded with ballots developed through Qualtrics (Provo, UT).

### WBSF

Samples were cooked as described earlier, chilled overnight at (2°–4°C), and sheared using the protocol outlined in the American Meat Science Association (AMSA, 2015) sensory guidelines. Six 1.27-cm-diameter cores were obtained parallel to the muscle fiber orientation using a manual coring device. Cores were sheared in the center of the core perpendicular to the muscle fiber orientation using a WBSF analyzer (G-R Elec. Mfg Co., Manhattan, KS) and measured in kilograms of force. The peak force was recorded for each core, and the 6 values were averaged for each steak.

### Volatile compound analysis

Volatile compound collection and gas chromatography-mass spectrometry analysis were conducted using the modified version of the methods described by Legako et al. (2016). Volatile compound analysis

was conducted on cooked samples ( $n = 96$ ) prepared as previously described. Immediately after cooking, six 1.27-cm-diameter cores were removed from the steaks perpendicular to the cooked surface using a manual coring device and minced with a coffee grinder (Coffee grinder, Mr. Coffee, Cleveland, OH). Five grams of the minced sample was weighed into a 20-mL gas chromatography vial and secured with a polytetrafluoroethylene septa and screw cap. Ten milliliters of an internal standard (1,2-dichlorobenzene; 1.306 mg/mL) was added to the vial, and the vial was loaded by a Gerstel automated sampler (MPS; Linthicum, MD). Analysis of volatile compounds was conducted using an Agilent 7890 gas chromatograph (Agilent Technologies, Santa Clara, CA) and 5977A mass selection detector (Agilent Technologies, Santa Clara, CA) equipped with a Gerstel automated sampler (MPS, Gerstel Inc., Linthicum, MD) according to the method outlined by Chail et al. (2017). Volatile compounds were extracted from samples via solid-phase microextraction and separated using a VF-5 ms capillary column. Volatile compound identity was initially determined by a mass spectral library (NIST MS Search 2.0,

National Institute of Standards and Technology, Gaithersburg MD). Compound identity was validated by comparison with retention times and ion fragmentation patterns to external standards (Sigma-Aldrich, Saint Louis, MO). Compounds consistently identified in each chromatogram were retained for treatment comparison. A 5-point external standard method was used for quantification. Standard reference compounds (Sigma-Aldrich, Saint Louis, MO) were injected (0.1  $\mu$ L) in solutions of pentane (late eluting compounds) or toluene (early eluting compounds) in splitless mode. Calibration curve slopes and intercepts were produced for each compound based on target area ratios and concentrations ratios between individual compounds and the internal standard. Unknown concentrations were determined in sample runs relative to the calibration curve slope, intercept, internal standard concentration, and internal standard ion area. Final values were calculated as nanograms of volatile compound extracted divided by sample weight in grams.

### Statistical analysis

All data were analyzed using the GLIMMIX procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC) as a factorial arrangement of a completely randomized design with diet, postmortem aging, and their interaction as fixed effects. Subprimal identification and harvest day were included as random effects. Final temperature was included as a covariate for trained sensory panel data, WBSF values, and cooked volatile compound data ( $P < 0.05$ ). Treatment least-squares means were separated with the PDIF option of SAS using a significance level of  $P \leq 0.05$ . Pearson correlation coefficients were generated between palatability and compositional traits using PROC CORR in SAS. Pearson correlation coefficients were considered significant at  $P < 0.05$ .

## Results and Discussion

### Proximate composition and pH

Results for the compositional data, pH, and objective color can be found in Table 2 for traits with no detectable interactions between diet and postmortem aging. Fat percentage differed ( $P < 0.01$ ) between diet designation, with grain-fed beef having a greater fat percentage. The fat content from the grass-fed samples was comparable to the expected fat content from US Department of Agriculture (USDA) Standard *longissimus*, while the fat content from the grain-fed samples was similar to the expected fat of a USDA Select *longissimus* (Corbin et al., 2015; Gredell et al., 2018). Previous findings suggest that grass-fed beef is leaner compared to grain-fed or conventionally raised beef (Melton et al., 1982; French et al., 2001). Similarly, many researchers have also reported that grass-fed cattle produce carcasses with less fat than grain-fed cattle (Bowling et al., 1977; Schroeder et al., 1980; Tatum et al., 1980; Dolezal et al., 1982). Fat percentage was not influenced by postmortem aging ( $P > 0.05$ ). Protein percentage was also similar between diets and across all postmortem aging periods ( $P > 0.05$ ). Diet also influenced pH ( $P = 0.01$ ), with grass-fed beef having greater pH than grain-fed samples. Previous reports have also shown that grass-fed beef had a greater pH than grain-finished beef (Muir et al., 1998; Ferguson, 2000). According to Muir et al. (1998), increased or high pH in grass-fed cattle may be the result of increased stress during slaughter since the animals are not used to handling through their feeding period. This was not an unexpected finding, according to Larick et al. (1987), since grass-fed cattle typically intake diets with lower net energy compared to grain-fed cattle. Postmortem aging did not affect pH ( $P > 0.05$ ).

**Table 2.** The effects of diet and postmortem aging on the least-squares means for pH, compositional, and objective color data from Australian *longissimus thoracis* ( $n = 96$ ; 12 per diet  $\times$  aging treatment combination)

| Attribute  | Diet              |                   | SEM  | Postmortem Aging, d |                    |                    |                    | SEM <sup>1</sup> | P      |        |                     |
|------------|-------------------|-------------------|------|---------------------|--------------------|--------------------|--------------------|------------------|--------|--------|---------------------|
|            | Grass             | Grain             |      | 35                  | 45                 | 55                 | 65                 |                  | Diet   | Aging  | Diet $\times$ Aging |
| Fat, %     | 2.05 <sup>b</sup> | 3.21 <sup>a</sup> | 0.19 | 2.71                | 2.69               | 2.75               | 2.38               | 0.28             | < 0.01 | 0.76   | 0.48                |
| Protein, % | 23.16             | 23.25             | 0.09 | 23.13               | 23.22              | 23.31              | 23.16              | 0.13             | 0.51   | 0.79   | 0.38                |
| pH         | 5.77 <sup>a</sup> | 5.71 <sup>b</sup> | 0.02 | 5.72                | 5.72               | 5.72               | 5.79               | 0.02             | 0.01   | 0.07   | 0.11                |
| $a^{*2}$   | 21.35             | 21.65             | 0.45 | 23.65 <sup>a</sup>  | 21.17 <sup>b</sup> | 20.06 <sup>b</sup> | 21.11 <sup>b</sup> | 0.64             | 0.64   | < 0.01 | 0.52                |
| $b^{*3}$   | 14.69             | 14.02             | 0.59 | 16.89               | 14.11              | 12.63              | 13.77              | 0.34             | 0.70   | < 0.01 | 0.79                |

<sup>1</sup>Pooled (largest) SE of least-squares (LS) means.

<sup>2</sup> $a^*$  (redness/greenness; positive values = red and negative values = green).

<sup>3</sup> $b^*$  (yellowness/blueness; positive values = yellow and negative values = blue).

<sup>ab</sup>LS means within a row and within a factor with the same superscript are not significantly different ( $P < 0.05$ ).

Postmortem aging influenced  $a^*$  and  $b^*$  values, but diet had no impact on either trait ( $P > 0.05$ ). Samples aged 35 d had greater ( $P < 0.05$ ) values for both traits compared to all other aging periods, which did not differ ( $P > 0.05$ ). Oliete et al. (2005) reported wet aging (7, 14, and 21 d) increased  $a^*$  and  $b^*$  values in vacuum-packaged *longissimus thoracis* steaks, which contradict these findings. Reverte et al. (2003) reported no difference in  $a^*$  and  $b^*$  values between grass- and grain-fed beef, which corresponds to our findings. However, Schroeder et al. (1980) reported that diet influenced  $a^*$  values, as beef that was produced on a higher-energy diet had lean with a brighter and redder color compared to grass-finished beef.

Table 3 presents least-squares means for moisture percentage and  $L^*$  values due to the interaction ( $P < 0.01$ ) between diet and postmortem aging. At 35 and 45 d postmortem, samples from grass-fed cattle had greater ( $P < 0.05$ ) moisture percentage than grain-fed samples; however, for samples aged 55 and 65 d postmortem, there was no difference ( $P > 0.05$ ) between moisture percentage between grass- and grain-fed samples. At 35, 45, and 65 d postmortem, grain-fed samples were lighter ( $P < 0.05$ ), as evidenced by greater  $L^*$  values, than the grain-fed samples at those aging periods. There was no difference in  $L^*$  values between grass- and grain-fed samples at 55 d postmortem.  $L^*$  values increased, suggesting the samples were lighter, through 55 d postmortem aging for grass-fed samples, and then declined at 65 d. However, grain-fed samples did not follow a consistent linear trend as postmortem aging period increased. Grain-fed samples aged 45 d postmortem had greater ( $P < 0.05$ )  $L^*$  values than any other treatment combination but did not differ ( $P > 0.05$ ) from grain-fed samples aged 65 d postmortem. Many unknown factors could be responsible for the lack of linear trend in  $L^*$  values, such as the age of the animal, sex, and breed type. Results from the literature suggest that grass-fed beef has darker color compared to grain-fed beef (Bennet et al., 1995;

Vestergaard et al., 2000; Yang et al., 2002; Garmyn et al., 2010), which generally agrees with the results of this study. Grass-fed cattle are sometimes fed longer to reach a similar or predetermined end-point as grain-fed animals and are consequently more mature, which could partially explain the darker lean color (Lanari et al., 2002; Garmyn et al., 2010). However, no documentation of chronological age was available, and carcass maturity was not assessed in the current study to support this theory. In fact, we believe the animals were likely similar in physiological age based on national average ossification scores for Meat Standards Australia graded carcasses designated as grass-fed or grain-fed. Ossification score, which is assessed much like skeletal maturity in the USDA quality grading system, serves as a measure of physiological maturity. According to MLA (2019), grass-fed and grain-fed carcasses have similar average ossification scores (170 vs. 160, respectively). As a result of the probable similarity in physiological age between the 2 diets, future discussion will focus more on diet than the possible age difference between cattle finished using the 2 diets.

### Trained sensory panel and WBSF

Table 4 illustrates the effects of diet and postmortem aging on flavor attributes, tenderness, and juiciness of *longissimus thoracis* samples. No interactions were observed ( $P > 0.05$ ) between diet and postmortem aging for any of the descriptive sensory panel attributes or WBSF. Beef flavor identity, fat-like, metallic, umami, bitter, and sweet were not influenced ( $P > 0.05$ ) by diet, postmortem aging, or their interaction. Diet influenced ( $P < 0.05$ ) several flavor attributes, including liver-like, rancid, grassy, and sour. Liver-like was the only flavor attribute that was detected more intensely ( $P < 0.05$ ) in grain-fed than grass-fed samples. Grass-fed samples had stronger ( $P < 0.05$ ) rancid, grassy, and sour flavors than grain-fed samples.

**Table 3.** The interactive effects of diet and postmortem aging on the least-squares means for moisture percentage and lightness ( $L^*$  values) from Australian *longissimus thoracis* ( $n = 96$ ; 12 per diet  $\times$  aging treatment combination)

| Attribute          | Grass, d           |                     |                      |                     | Grain, d             |                    |                      |                      | SEM <sup>1</sup> | P      |        |                     |
|--------------------|--------------------|---------------------|----------------------|---------------------|----------------------|--------------------|----------------------|----------------------|------------------|--------|--------|---------------------|
|                    | 35                 | 45                  | 55                   | 65                  | 35                   | 45                 | 55                   | 65                   |                  | Diet   | Aging  | Diet $\times$ Aging |
| Moisture, %        | 73.30 <sup>b</sup> | 74.26 <sup>a</sup>  | 72.99 <sup>bcd</sup> | 73.87 <sup>ab</sup> | 71.99 <sup>de</sup>  | 72.17 <sup>c</sup> | 73.17 <sup>bc</sup>  | 72.37 <sup>cde</sup> | 0.33             | < 0.01 | 0.32   | < 0.01              |
| $L^*$ <sup>2</sup> | 37.47 <sup>f</sup> | 41.08 <sup>de</sup> | 43.94 <sup>bc</sup>  | 40.37 <sup>e</sup>  | 41.68 <sup>cde</sup> | 47.16 <sup>a</sup> | 43.67 <sup>bcd</sup> | 46.37 <sup>ab</sup>  | 0.33             | < 0.01 | < 0.01 | < 0.01              |

<sup>1</sup>Pooled (largest) SE of least-squares (LS) means.

<sup>2</sup> $L^*$  (brightness; 0 = black and 100 = white).

<sup>a-e</sup>LS means within a row with the same superscript are not significantly different ( $P < 0.05$ ).

**Table 4.** The effects of diet and postmortem aging on the least-squares means for flavor attributes, tenderness, and juiciness from Australian *longissimus thoracis* ( $n = 96$ ; 12 per diet  $\times$  aging treatment combination)

| Attributes <sup>1</sup> | Diet              |                   | SEM <sup>2</sup> | Postmortem Aging, d |                   |                   |                    | SEM <sup>2</sup> | <i>P</i> |        |                     |
|-------------------------|-------------------|-------------------|------------------|---------------------|-------------------|-------------------|--------------------|------------------|----------|--------|---------------------|
|                         | Grass             | Grain             |                  | 35                  | 45                | 55                | 65                 |                  | Diet     | Aging  | Diet $\times$ Aging |
| <b>Beef flavor</b>      | 46.6              | 46.3              | 1.7              | 46.6                | 47.9              | 46.7              | 44.8               | 1.8              | 0.72     | 0.06   | 0.41                |
| <b>Bloody/Serumy</b>    | 8.8               | 8.2               | 1.1              | 8.5 <sup>ab</sup>   | 9.6 <sup>a</sup>  | 7.4 <sup>b</sup>  | 8.5 <sup>ab</sup>  | 1.1              | 0.19     | 0.03   | 0.22                |
| <b>Fat-like</b>         | 11.6              | 11.8              | 1.1              | 11.9                | 11.9              | 11.5              | 11.5               | 1.2              | 0.65     | 0.73   | 0.38                |
| <b>Liver-like</b>       | 5.1 <sup>y</sup>  | 7.7 <sup>x</sup>  | 0.8              | 4.2 <sup>c</sup>    | 5.8 <sup>bc</sup> | 7.0 <sup>ab</sup> | 8.4 <sup>a</sup>   | 0.9              | < 0.01   | < 0.01 | 0.43                |
| <b>Metallic</b>         | 7.4               | 6.9               | 0.9              | 6.5                 | 6.9               | 7.3               | 7.8                | 0.9              | 0.22     | 0.12   | 0.68                |
| <b>Rancid</b>           | 9.3 <sup>x</sup>  | 7.2 <sup>y</sup>  | 1.1              | 5.9 <sup>c</sup>    | 7.8 <sup>b</sup>  | 8.5 <sup>b</sup>  | 10.8 <sup>a</sup>  | 1.2              | 0.01     | < 0.01 | 0.84                |
| <b>Grassy</b>           | 9.8 <sup>x</sup>  | 7.7 <sup>y</sup>  | 1.2              | 7.1 <sup>b</sup>    | 8.8 <sup>a</sup>  | 9.4 <sup>a</sup>  | 9.8 <sup>a</sup>   | 1.2              | < 0.01   | 0.02   | 0.78                |
| <b>Umami</b>            | 13.1              | 12.6              | 1.8              | 13.3                | 13.6              | 12.9              | 11.7               | 1.9              | 0.35     | 0.08   | 0.59                |
| <b>Sour</b>             | 7.0 <sup>x</sup>  | 6.3 <sup>y</sup>  | 1.3              | 6.2                 | 6.5               | 6.5               | 7.4                | 1.3              | 0.04     | 0.13   | 0.50                |
| <b>Bitter</b>           | 3.5               | 2.9               | 0.7              | 2.8                 | 3.4               | 3.2               | 3.4                | 0.7              | 0.06     | 0.56   | 0.06                |
| <b>Sweet</b>            | 1.4               | 1.3               | 0.4              | 1.7                 | 1.4               | 1.2               | 1.2                | 0.5              | 0.46     | 0.07   | 0.96                |
| <b>Juiciness</b>        | 46.1 <sup>x</sup> | 41.3 <sup>y</sup> | 2.3              | 43.0 <sup>b</sup>   | 48.1 <sup>a</sup> | 41.3 <sup>b</sup> | 42.3 <sup>b</sup>  | 2.6              | < 0.01   | 0.01   | 0.22                |
| <b>Tenderness</b>       | 53.9              | 53.6              | 1.5              | 50.3 <sup>c</sup>   | 56.9 <sup>a</sup> | 53.4 <sup>b</sup> | 54.4 <sup>ab</sup> | 1.5              | 0.78     | < 0.01 | 0.17                |
| <b>WBSF, kg</b>         | 2.29              | 2.06              | 0.11             | 2.50                | 1.99              | 2.18              | 2.03               | 0.15             | 0.14     | 0.08   | 0.75                |

<sup>1</sup>Sensory scores: 0 = slight, 50 = moderate, and 100 = strong.

<sup>2</sup>Pooled (largest) SE of least-squares (LS) means.

<sup>abc</sup>LS means within a row lacking a common superscript differ ( $P < 0.05$ ) due to postmortem aging.

<sup>xy</sup>LS means within row lacking a common superscript differ ( $P < 0.05$ ) due to diet.

WBSF = Warner-Bratzler shear force.

Grain-fed beef was reported to have stronger livery flavor, which is considered a common off-flavor associated with beef (Eilers et al., 1994; Morris et al., 1997; Campo et al., 1999). Melton et al. (1982) found that liver-flavor intensity increased when feeding cattle up to 86 d on corn-based diets compared to 0 d on a corn-based diet. As expected, grassy flavor was stronger in grass- than grain-fed samples, as was rancid and sour. Daley et al. (2010) believed that the variation in fatty acid content of grass-fed beef can be responsible for a distinct grassy flavor. French et al. (2001) thought that the green-hay flavor in beef was likely due to decreased unsaturated fats and conjugated linoleic acids in grass-finished beef compared to grain-fed beef.

Postmortem aging influenced ( $P < 0.05$ ) several flavor attributes, including bloody/serumy, liver-like, rancid, and grassy. Bloody/serumy flavor was more intense ( $P < 0.05$ ) in samples aged 45-d compared to 55-d samples but did not differ ( $P > 0.05$ ) from any other aging period. For all other flavor attributes influenced by postmortem aging, panelists generally detected stronger flavor as postmortem aging increased. The liver-like flavor was stronger ( $P < 0.05$ ) in samples aged 65 d than samples aged 45 and 35 d, which were similar ( $P > 0.05$ ). Rancid flavor was stronger ( $P < 0.05$ ) in samples aged 65 d compared to any other postmortem aging period; samples aged

45 and 55 d were similar ( $P > 0.05$ ) and intermediate, and 35-d samples had the lowest rancid flavor detection. The grassy flavor was weaker ( $P < 0.05$ ) in samples aged 35 d compared to all other postmortem aging periods, which did not differ ( $P > 0.05$ ).

Lipid oxidation is limited by endogenous antioxidant mechanisms in living muscle (Sies, 1985), but effectiveness of these antioxidants declines as postmortem aging time increases (Monahan, 2000) resulting in increased lipid oxidation (Hughes et al., 2015). Since lipid oxidation has been linked with rancid flavor (Mottram and Edwards, 1983; Asghar, 1988), this would explain why the rancid flavor grew stronger as postmortem aging increased in the current study. Liver-like flavor also increased as postmortem aging increased in the current study, which is supported by Campo et al. (1999), who found that livery flavor increased with extended postmortem aging.

Diet and postmortem aging both influenced ( $P < 0.05$ ) juiciness. Grass-fed samples were juicier ( $P < 0.05$ ) compared to grain-fed samples, and samples aged 45 d were juicier than samples aged 35, 55, and 65 d, which were all similar ( $P > 0.05$ ). Grass-fed samples may have been considered juicier due to the initial juiciness, which is attributed more to moisture, since grass-fed samples had greater moisture than grain-fed samples. Grass-fed samples also had greater pH, which could lead to greater water-holding capacity and result in greater juiciness.

However, Garmyn et al. (2010) did not observe any differences in juiciness between concentrate- and grass-finished beef, which does not support the current findings. Lepper-Blilie et al. (2016) did not observe any differences in juiciness between beef loin samples aged 14 to 49 d postmortem. Despite the increased juiciness in samples aged 45 d in the current study, no other differences were observed in juiciness due to aging.

Finally, sensory tenderness was not influenced by diet ( $P > 0.05$ ) but was impacted ( $P < 0.01$ ) by post-mortem aging. However, WBSF was not influenced ( $P > 0.05$ ) by diet or postmortem aging. Likewise, previous results have shown no difference in WBSF in beef from cattle finished on pasture compared to grain when harvested at a similar age or degree of finish (French et al., 2001; Duckett et al., 2013). However, Garmyn et al. (2010) reported that grass-fed samples were tougher, both instrumentally and when assessed by trained panelists, which contradicts the current findings. Those samples were aged 10 d postmortem, whereas the current samples were aged a minimum of 35 d postmortem. It is well documented that tenderness increases as postmortem aging increases (Gruber et al., 2006; Lepper-Blilie et al., 2016), especially through 21 d postmortem when several proteins, which are thought to contribute to tenderization, are degraded (Taylor et al., 1995; Robson et al., 1997). When focusing on the aging periods that are relevant to the current study (35–49 d), Lepper-Blilie et al. (2016) did not observe any differences in WBSF or sensory tenderness scores. However, regardless of diet or postmortem aging period, samples in the current study would all be classified as very tender (WBSF  $< 3.9$  kg) as they were below accepted threshold values for tenderness (Miller et al., 2001; Shackelford et al., 2001; ASTM, 2011).

### Cooked volatile flavor compounds

The development of distinctive flavor of cooked meat is ascribed to volatile compounds produced during heating as a result of the Maillard reaction, lipid oxidation, and interaction between the products of the Maillard reaction, lipid oxidation, and thermal degradation of thiamine (MacLeod, 1998). Tables 5 and 6 show the main effects of diet and postmortem aging, respectively, on the quantities of volatile compounds from grass- and grain-fed Australian beef *longissimus thoracis*, whereas compounds influenced by interactive effects can be found in Table 7. Diet had a limited effect on the concentration of volatile compounds (Table 5). However, content of acetic acid and hexanal were each greater ( $P < 0.05$ ) in grain-fed beef. Hexanal is

**Table 5.** The effect of diet on the least-squares means for volatile flavor compounds (ng/g of sample) from Australian *longissimus thoracis* ( $n = 96$ ; 48 per diet)

| Volatile Compounds          | Diet               |                    | SEM <sup>1</sup> | P<br>Diet |
|-----------------------------|--------------------|--------------------|------------------|-----------|
|                             | Grain              | Grass              |                  |           |
| <b>n-Aldehydes</b>          |                    |                    |                  |           |
| Acetaldehyde                | 10.83              | 8.96               | 0.69             | 0.06      |
| Pentanal                    | 2.73               | 2.96               | 0.24             | 0.50      |
| Hexanal                     | 63.41 <sup>a</sup> | 52.63 <sup>b</sup> | 3.36             | 0.02      |
| Heptanal                    | 17.01              | 20.65              | 1.62             | 0.11      |
| Octanal                     | 4.10               | 4.73               | 0.41             | 0.28      |
| Nonanal                     | 12.82              | 15.32              | 1.36             | 0.19      |
| Decanal                     | 16.23              | 19.34              | 1.27             | 0.09      |
| Dodecanal                   | 12.74              | 12.39              | 2.39             | 0.92      |
| <b>Strecker Aldehydes</b>   |                    |                    |                  |           |
| 3-methylbutanal             | 85.58              | 81.60              | 7.97             | 0.72      |
| 2-methylbutanal             | 56.20              | 53.90              | 5.72             | 0.78      |
| Benzaldehyde                | 58.30              | 59.82              | 2.20             | 0.62      |
| Phenylacetaldehyde          | 4.52               | 4.24               | 0.25             | 0.42      |
| <b>Ketones</b>              |                    |                    |                  |           |
| 2-propanone                 | 69.47              | 71.57              | 4.15             | 0.72      |
| 2-butanone                  | 50.08              | 49.07              | 3.37             | 0.83      |
| 2-pentanone                 | 0.55               | 0.52               | 0.03             | 0.55      |
| 2-heptanone                 | 1.01               | 0.94               | 0.03             | 0.11      |
| <b>Sulfur containing</b>    |                    |                    |                  |           |
| Dimethyl disulfide          | 0.17               | 0.14               | 0.02             | 0.28      |
| Dimethyl sulfide            | 4.26               | 3.88               | 0.23             | 0.25      |
| Carbon disulfide            | 4.38               | 4.69               | 0.26             | 0.40      |
| <b>Thiols</b>               |                    |                    |                  |           |
| Methanethiol                | 13.72              | 11.61              | 0.89             | 0.10      |
| Methional                   | 2.53               | 2.87               | 0.13             | 0.06      |
| <b>Pyrazines</b>            |                    |                    |                  |           |
| Methyl pyrazine             | 4.30               | 3.96               | 0.47             | 0.61      |
| 2,5-dimethylpyrazine        | 6.94               | 6.81               | 0.79             | 0.90      |
| Trimethylpyrazine           | 1.31               | 1.36               | 0.15             | 0.83      |
| <b>Alkanes</b>              |                    |                    |                  |           |
| Octane                      | 11.10              | 12.21              | 1.32             | 0.55      |
| <b>Alkenes</b>              |                    |                    |                  |           |
| 1-Octene                    | 2.42               | 2.34               | 0.23             | 0.81      |
| <b>Alcohols</b>             |                    |                    |                  |           |
| Ethanol                     | 43.68              | 37.33              | 7.72             | 0.56      |
| 1-penten-3-ol               | 5.00               | 4.58               | 0.56             | 0.59      |
| 1-pentanol                  | 5.35               | 5.37               | 0.38             | 0.97      |
| 1-hexanol                   | 2.75               | 2.84               | 0.21             | 0.74      |
| 1-octanol                   | 30.07              | 30.10              | 0.84             | 0.98      |
| <b>Carboxylic Acids</b>     |                    |                    |                  |           |
| Acetic acid                 | 10.60 <sup>a</sup> | 8.94 <sup>b</sup>  | 0.49             | 0.02      |
| Nonanoic acid               | 0.39               | 0.38               | 0.04             | 0.73      |
| <b>Esters</b>               |                    |                    |                  |           |
| Butanoic acid, methyl ester | 0.33               | 0.10               | 0.13             | 0.21      |
| Hexanoic acid, methyl ester | 0.64               | 0.28               | 0.16             | 0.10      |
| Octanoic acid, methyl ester | 0.96               | 0.90               | 0.04             | 0.24      |

<sup>1</sup>Pooled (largest) SE of least-squares means.

<sup>ab</sup>Means within row lacking common superscript differ ( $P < 0.05$ ).

**Table 6.** The effect of postmortem aging on the least-squares means for volatile flavor compounds (ng/g of sample) from Australian *longissimus thoracis* ( $n = 96$ ; 24 per aging period)

| Volatile Compounds          | Postmortem aging, d |                     |                     |                     | SEM <sup>1</sup> | P<br>Aging |
|-----------------------------|---------------------|---------------------|---------------------|---------------------|------------------|------------|
|                             | 35                  | 45                  | 55                  | 65                  |                  |            |
| <b>n-Aldehydes</b>          |                     |                     |                     |                     |                  |            |
| Acetaldehyde                | 8.56 <sup>b</sup>   | 15.94 <sup>a</sup>  | 16.01 <sup>a</sup>  | 7.76 <sup>b</sup>   | 2.05             | < 0.01     |
| Pentanal                    | 1.93 <sup>b</sup>   | 5.69 <sup>a</sup>   | 5.43 <sup>a</sup>   | 1.61 <sup>b</sup>   | 1.09             | 0.01       |
| Hexanal                     | 32.58 <sup>b</sup>  | 104.55 <sup>a</sup> | 107.0 <sup>a</sup>  | 25.34 <sup>b</sup>  | 16.64            | < 0.01     |
| Heptanal                    | 11.78 <sup>b</sup>  | 38.73 <sup>a</sup>  | 13.32 <sup>b</sup>  | 19.69 <sup>b</sup>  | 4.52             | < 0.01     |
| Octanal                     | 3.42 <sup>b</sup>   | 6.57 <sup>a</sup>   | 5.57 <sup>a</sup>   | 3.03 <sup>b</sup>   | 0.61             | < 0.01     |
| Nonanal                     | 11.89 <sup>b</sup>  | 22.86 <sup>a</sup>  | 18.08 <sup>a</sup>  | 10.79 <sup>b</sup>  | 2.17             | < 0.01     |
| Decanal                     | 23.72 <sup>b</sup>  | 40.35 <sup>a</sup>  | 34.77 <sup>a</sup>  | 24.02 <sup>b</sup>  | 2.80             | < 0.01     |
| Dodecanal                   | 17.83               | 14.98               | 7.43                | 10.01               | 3.42             | 0.12       |
| <b>Strecker Aldehydes</b>   |                     |                     |                     |                     |                  |            |
| 3-Methylbutanal             | 30.53 <sup>b</sup>  | 123.81 <sup>a</sup> | 129.34 <sup>a</sup> | 50.67 <sup>b</sup>  | 11.39            | < 0.01     |
| 2-Methylbutanal             | 18.79 <sup>b</sup>  | 82.97 <sup>a</sup>  | 85.45 <sup>a</sup>  | 32.95 <sup>b</sup>  | 8.18             | < 0.01     |
| Benzaldehyde                | 60.05 <sup>ab</sup> | 52.60 <sup>b</sup>  | 57.31 <sup>b</sup>  | 66.29 <sup>a</sup>  | 3.15             | 0.02       |
| Phenylacetaldehyde          | 3.84 <sup>b</sup>   | 3.46 <sup>b</sup>   | 3.87 <sup>b</sup>   | 6.36 <sup>a</sup>   | 0.36             | < 0.01     |
| <b>Ketones</b>              |                     |                     |                     |                     |                  |            |
| 2-Propanone                 | 55.55 <sup>b</sup>  | 77.05 <sup>a</sup>  | 80.11 <sup>a</sup>  | 69.36 <sup>ab</sup> | 5.94             | 0.02       |
| 2-Butanone                  | 37.29 <sup>b</sup>  | 58.71 <sup>a</sup>  | 61.57 <sup>a</sup>  | 40.72 <sup>b</sup>  | 4.82             | < 0.01     |
| 2-Pentanone                 | 0.40 <sup>b</sup>   | 0.66 <sup>a</sup>   | 0.62 <sup>a</sup>   | 0.45 <sup>b</sup>   | 0.06             | < 0.01     |
| 2-Heptanone                 | 0.98                | 0.92                | 0.98                | 1.04                | 0.05             | 0.31       |
| <b>Sulfur Containing</b>    |                     |                     |                     |                     |                  |            |
| Dimethyl disulfide          | 0.09 <sup>b</sup>   | 0.16 <sup>b</sup>   | 0.26 <sup>a</sup>   | 0.11 <sup>b</sup>   | 0.03             | < 0.01     |
| Dimethyl sulfide            | 3.29 <sup>b</sup>   | 4.88 <sup>a</sup>   | 4.62 <sup>a</sup>   | 3.51 <sup>b</sup>   | 0.33             | < 0.01     |
| Carbon disulfide            | 5.20 <sup>a</sup>   | 3.57 <sup>b</sup>   | 4.25 <sup>ab</sup>  | 5.13 <sup>a</sup>   | 0.37             | < 0.01     |
| <b>Thiols</b>               |                     |                     |                     |                     |                  |            |
| Methanethiol                | 9.46 <sup>b</sup>   | 13.01 <sup>a</sup>  | 14.74 <sup>a</sup>  | 13.45 <sup>a</sup>  | 1.27             | 0.02       |
| Methional                   | 2.20 <sup>b</sup>   | 2.81 <sup>a</sup>   | 3.05 <sup>a</sup>   | 2.76 <sup>a</sup>   | 0.18             | < 0.01     |
| <b>Pyrazines</b>            |                     |                     |                     |                     |                  |            |
| Methyl-pyrazine             | 2.63 <sup>b</sup>   | 5.05 <sup>a</sup>   | 5.08 <sup>a</sup>   | 3.75 <sup>ab</sup>  | 0.67             | 0.03       |
| 2,5-dimethylpyrazine        | 4.55 <sup>b</sup>   | 8.32 <sup>a</sup>   | 8.33 <sup>a</sup>   | 6.29 <sup>ab</sup>  | 1.13             | 0.05       |
| Trimethylpyrazine           | 1.02 <sup>b</sup>   | 1.06 <sup>b</sup>   | 1.53 <sup>ab</sup>  | 1.73 <sup>a</sup>   | 0.21             | 0.05       |
| <b>Alkanes</b>              |                     |                     |                     |                     |                  |            |
| Octane                      | 9.57                | 13.12               | 12.86               | 11.07               | 1.89             | 0.50       |
| <b>Alkenes</b>              |                     |                     |                     |                     |                  |            |
| 1-Octene                    | 2.43                | 2.36                | 2.23                | 2.50                | 0.35             | 0.94       |
| <b>Alcohols</b>             |                     |                     |                     |                     |                  |            |
| Ethanol                     | 25.23 <sup>b</sup>  | 27.82 <sup>b</sup>  | 66.10 <sup>a</sup>  | 42.87 <sup>ab</sup> | 10.80            | 0.04       |
| 1-Penten-3-ol               | 1.49 <sup>b</sup>   | 7.47 <sup>a</sup>   | 7.42 <sup>a</sup>   | 2.79 <sup>b</sup>   | 0.80             | < 0.01     |
| 1-Pentanol                  | 3.08 <sup>b</sup>   | 7.43 <sup>a</sup>   | 7.21 <sup>a</sup>   | 3.73 <sup>b</sup>   | 0.55             | < 0.01     |
| 1-Hexanol                   | 2.70                | 3.27                | 2.51                | 2.69                | 0.29             | 0.29       |
| 1-Octanol                   | 29.79               | 29.92               | 28.17               | 32.47               | 1.20             | 0.08       |
| <b>Carboxylic Acids</b>     |                     |                     |                     |                     |                  |            |
| Acetic acid                 | 7.06 <sup>b</sup>   | 8.99 <sup>b</sup>   | 11.85 <sup>a</sup>  | 11.16 <sup>a</sup>  | 0.70             | < 0.01     |
| Nonanoic acid               | 0.29 <sup>b</sup>   | 0.51 <sup>a</sup>   | 0.44 <sup>a</sup>   | 0.29 <sup>b</sup>   | 0.05             | < 0.01     |
| <b>Esters</b>               |                     |                     |                     |                     |                  |            |
| Butanoic acid, methyl ester | 0.07                | 0.17                | 0.47                | 0.15                | 0.18             | 0.42       |
| Hexanoic acid, methyl ester | 0.27                | 0.32                | 0.58                | 0.67                | 0.23             | 0.52       |
| Octanoic acid, methyl ester | 1.07 <sup>a</sup>   | 0.80 <sup>b</sup>   | 0.81 <sup>b</sup>   | 1.04 <sup>a</sup>   | 0.05             | < 0.01     |

<sup>1</sup> Pooled (largest) SE of least-squares means.<sup>abc</sup> Means within row lacking common superscript differ ( $P < 0.05$ ).

**Table 7.** The interactive effects of diet and postmortem aging on the least-squares means for volatile flavor compounds (ng/g of sample) from Australian *longissimus thoracis* ( $n = 96$ ; 12 per diet  $\times$  aging treatment combination)

| Volatile Compound                      | Grain                |                      |                      |                      | Grass               |                     |                      |                     | SEM <sup>1</sup> | Diet $\times$ Aging |
|--|----------------------|----------------------|----------------------|----------------------|---------------------|---------------------|----------------------|---------------------|------------------|---------------------|
|  | 35 d                 | 45 d                 | 55 d                 | 65 d                 | 35 d                | 45 d                | 55 d                 | 65 d                |                  |                     |
| <b>2,3-Butanedione</b>                 | 59.91 <sup>bcd</sup> | 152.27 <sup>a</sup>  | 86.02 <sup>b</sup>   | 46.04 <sup>cd</sup>  | 37.78 <sup>cd</sup> | 44.87 <sup>cd</sup> | 64.80 <sup>bc</sup>  | 24.46 <sup>d</sup>  | 14.41            | < 0.01              |
| <b>2,3-Pentanedione</b>                | 0.08 <sup>a</sup>    | 0.04 <sup>ab</sup>   | 0.10 <sup>a</sup>    | 0.07 <sup>a</sup>    | 0.07 <sup>ab</sup>  | 0.08 <sup>a</sup>   | 0.01 <sup>b</sup>    | 0.07 <sup>ab</sup>  | 0.03             | 0.04                |
| <b>3-Hydroxy-2-butanone</b>            | 96.58 <sup>bc</sup>  | 200.19 <sup>a</sup>  | 119.34 <sup>b</sup>  | 80.74 <sup>bcd</sup> | 56.72 <sup>cd</sup> | 65.62 <sup>cd</sup> | 100.07 <sup>bc</sup> | 41.83 <sup>d</sup>  | 17.50            | < 0.01              |
| <b>1-Octen-3-ol</b>                    | 3.61 <sup>b</sup>    | 2.60 <sup>cd</sup>   | 2.22 <sup>d</sup>    | 4.54 <sup>a</sup>    | 2.81 <sup>cd</sup>  | 2.19 <sup>d</sup>   | 2.57 <sup>cd</sup>   | 3.26 <sup>bc</sup>  | 0.28             | 0.03                |
| <b>2-Pentyl furan</b>                  | 0.68 <sup>ab</sup>   | 0.49 <sup>abcd</sup> | 0.49 <sup>abcd</sup> | 0.71 <sup>a</sup>    | 0.29 <sup>d</sup>   | 0.37 <sup>cd</sup>  | 0.55 <sup>abc</sup>  | 0.46 <sup>bcd</sup> | 0.08             | 0.05                |
| <b>2-Ethyl-3,5/6-dimethyl pyrazine</b> | 1.67 <sup>d</sup>    | 2.16 <sup>cd</sup>   | 2.16 <sup>cd</sup>   | 3.26 <sup>abc</sup>  | 2.18 <sup>cd</sup>  | 4.17 <sup>a</sup>   | 2.82 <sup>abcd</sup> | 2.50 <sup>bcd</sup> | 0.59             | 0.04                |

<sup>1</sup>Pooled (largest) SE of least-squares means.

<sup>abc</sup>Means within row lacking common superscript differ ( $P < 0.05$ ).

a primary indicator of lipid oxidation and has previously been determined to be greater in grain-finished beef compared with grass-finished (Chail et al., 2016). Grass-finished beef has been cited to have greater content of endogenous antioxidants (Realini et al., 2004; Descalzo et al., 2007). Ultimately greater antioxidant activity may have influenced rates of lipid oxidation and depressed expression of hexanal in the grass-fed beef of this study.

Multiple n-aldehydes and lipid-derived ketones were influenced ( $P < 0.05$ ) by postmortem aging (Table 6). For all n-aldehydes except heptanal, samples aged 45 and 55 d postmortem did not differ ( $P > 0.05$ ) and were greater ( $P < 0.05$ ) in concentration than samples aged 35 and 65 d postmortem, which were also similar. Heptanal concentration was greater ( $P < 0.05$ ) in samples aged 45 d than any other postmortem aging period, which were all similar ( $P > 0.05$ ). The aldehydes are products of lipid oxidation, and they are produced during the oxidative degradation of unsaturated fatty acids (Grosch, 1982; Frankel, 1991). Watanabe et al. (2015) also observed increased quantity of certain n-aldehydes due to postmortem aging. Heptanal, octane, and hexanal increased significantly from 2 to 30 d postmortem, while octanal, nonanal, 2-heptenal, 2-octenal, 2,4-decadienal, 1-octanol, and 1-octen-3-ol were notably higher, but only numerically (Watanabe et al., 2015). Past studies have referred to aldehydes as a cause of off-odor (Ahn and Kim, 1998; Ahn and Lee, 2002). Dietze et al. (2007) reported that aldehydes were responsible for off-flavor, and others described these compounds as responsible for rancidity (Ahn, 2002; Byrne et al., 2002; Campo et al., 2006). Samples aged 45 and 55 d postmortem in the current study were scored as more rancid than samples aged 35 d, which could be attributed to the higher concentration of these n-aldehydes. However, 65-d

samples had the strongest rancid scores during descriptive flavor analysis, which does not align with the lower concentration of n-aldehydes at 65 d. Shahidi and Pegg (1994) outlined that n-aldehydes, such as hexanal, may be degraded into smaller lipid oxidation products over time. Likewise, more recent work within our group further documents a lowering of n-aldehydes as aging progresses (Legako et al., 2018).

All measured ketones, except 2-heptanone ( $P = 0.31$ ), were influenced ( $P \leq 0.02$ ) by postmortem aging. In general, these compounds followed a consistent trend to a majority of the n-aldehydes. Both 2-butanone and 2-pentanone of samples aged 45 and 55 d postmortem did not differ ( $P > 0.05$ ) and were greater ( $P < 0.05$ ) in concentration than samples aged 35 and 65 d postmortem, which were also similar ( $P > 0.05$ ). 2-propanone was lower ( $P < 0.05$ ) in content at 35 d compared with 45 and 55 d, but 65-d samples were considered similar ( $P > 0.05$ ) to all other aging durations.

Other volatile lipid oxidation products—including 1-penten-3-ol, 1-pentanol, nonanoic acid, octanoic acid, and methyl ester—from samples aged 45 and 55 d postmortem did not differ ( $P > 0.05$ ) and were greater ( $P < 0.05$ ) in concentration than samples aged 35 and 65 d postmortem. As previously stated, this general response to aging was observed for several lipid oxidation volatile products. Sensory results indicate that 65-d samples were rated most highly for rancid and lowest for beef flavor identity. It would therefore be expected that lipid oxidation products would be greatest for 65 d. The measured lipid oxidation volatile compounds are not in alignment with this expectation. It is unclear whether this is due to further degradation of the measured volatile compounds, as described earlier, or some other unknown factor.

Aging also influenced content of acetic acid and ethanol ( $P \leq 0.04$ ). Acetic acid was increased

( $P < 0.05$ ) for 55- and 65-d samples. Likewise, ethanol was increased ( $P < 0.05$ ) for 55- and 65-d samples, but ethanol content at 65 d was comparable ( $P > 0.05$ ) to both 35- and 45-d samples. Acetic acid and ethanol are known byproducts of microbial growth. Prior work has indicated that ethanol may be increased during postmortem aging with microbial growth as the likely source (Ismail et al., 2008).

Strecker aldehydes have been reported to generate characteristic aroma notes, like butter, floral, sweet, or toasted odors (Stahnke, 1994; Belitz et al., 2004). In general, Strecker aldehydes impart desirable flavor characteristics. Multiple Strecker aldehydes were influenced ( $P \leq 0.05$ ) by postmortem aging, including 3-methyl butanal, 2-methyl butanal, benzaldehyde, and phenylacetaldehyde. Samples aged 45 and 55 d postmortem did not differ ( $P > 0.05$ ) for 3- and 2-methyl butanal content and were each greater ( $P < 0.05$ ) than samples aged 35 and 65 d postmortem, which were also similar ( $P > 0.05$ ). For benzaldehyde, 65-d samples had greater ( $P < 0.05$ ) concentration than samples aged 45 and 55 d postmortem, but 35-d samples did not differ from any other aging period ( $P > 0.05$ ). For phenylacetaldehyde, 65-d samples had greater ( $P < 0.05$ ) concentration than all other aging points, which were similar ( $P > 0.05$ ) to each other. Postmortem proteolysis during the aging process releases free amino acids as precursors to these volatile compounds. It was expected that Strecker aldehyde volatile quantities would vary in response to postmortem aging and release of amino acids. It is unclear, at this time, why at 65 d postmortem there was a lowering of 3- and 2-methyl butanal, compared with at 45 and 55 d.

Similar to Strecker aldehydes, sulfur-containing volatile compounds were influenced by aging ( $P \leq 0.02$ ). Dimethyl sulfide was greater ( $P < 0.05$ ) in samples aged 45 and 55 d than samples aged 35 and 65 d postmortem. Dimethyl disulfide was greatest ( $P < 0.05$ ) at 55 d compared with all other durations. For methanethiol and methional, concentrations increased over time with 35-d samples having lower ( $P < 0.05$ ) content compared with all subsequent aging duration. Sulfur-containing compounds, like dimethyl sulfide and methanethiol, were reported by Gasser and Grosch (1990) to contribute to meaty flavor notes. Release of sulfur-containing amino acids, such as methionine and cysteine, from proteolysis is a likely precursor to these sulfur-containing volatile compounds.

Several pyrazine compounds were influenced ( $P < 0.01$ ) by postmortem aging. Methyl-pyrazine and 2,5-dimethyl pyrazine were each lower ( $P < 0.05$ ) in content at 35 d compared with 45 and 55 d, but 65-d

samples were considered similar ( $P > 0.05$ ) to all other aging durations. Trimethylpyrazine content was greatest ( $P < 0.05$ ) for 65-d samples, but 65 d was similar ( $P > 0.05$ ) to 55 d. Watanabe et al. (2015) also observed increased quantities of some pyrazines during postmortem aging from 2 to 30 d postmortem. The formation of pyrazines results from the condensation of two  $\alpha$ -aminocarbonyls, which are derived from the Strecker reaction between an amino acid and  $\alpha$ -carbonyl compounds (Shahidi, 1998). According to Watanabe et al. (2015), the increase of some pyrazines during postmortem aging could be explained by increased free amino acids during storage and high cooking temperatures, which ultimately leads to their production through the Maillard reaction.

Compounds influenced by interactive effects of diet and postmortem aging can be found in Table 8. An interaction was observed ( $P < 0.05$ ) for 2,3-butanedione and 3-hydroxy-2-butanone. Both compounds followed similar trends in which grain-fed samples aged 45 d had the highest concentration compared to all other treatment combinations. For 2,3-butanedione, samples aged 35 and 65 d from both grass- and grain-fed samples had lower ( $P < 0.05$ ) concentration than grain-fed samples aged 45 and 55 d ( $P > 0.05$ ). Within each aging period, grass- and grain-fed samples had similar concentration of both compounds; however, this was not the case in samples aged 45 d, in which both compounds were detected in greater ( $P < 0.05$ ) concentration in grain-fed than in grass-fed samples. According to Hurrell (1982), these compounds arise from the 2,3-enolization pathways and are intermediates of the Maillard reaction. It is unclear why these compounds were differentiated only at 45 d. However, in prior work these 2 Maillard-reaction-derived compounds were determined to be greater in grain-finished beef compared to other forage-finished beef types (Chail et al., 2016).

Three lipid-oxidation-derived compounds, 2,3-pentanedione, 1-octen-3-ol, and 2-pentyl furan, were also influenced by interacting effects of diet and age ( $P \leq 0.05$ ). For 1-octen-3-ol, 65-d grain-finished samples had the greatest ( $P < 0.05$ ) content compared with all other diet and aging combinations. This volatile is produced through the oxidation of linoleic acid and has been related with liver-like flavor (Ullrich and Grosch, 1987; Hodgen et al., 2006; Yancey et al., 2006; Calkins and Hodgen, 2007). In this study, liver-like flavor was greater in 65-d samples and in grain-fed samples. It is plausible then, in agreement with the cited works, that lipid oxidation and 1-octen-3-ol had some influence on liver-like flavor in this study.

## Conclusions

Results from this study show that extended aging did not impact beef flavor identity and umami; however, it should be noted that certain off-flavors, especially liver-like and rancid, grew stronger as post-mortem aging time was extended. Flavor attributes such as rancid, grassy, and sour were stronger in grass- than grain-fed samples, but grain-fed samples had a stronger liver-like flavor. Aging influenced both overall tenderness and juiciness, but typically not in a linear manner. A majority of the flavor volatile compounds were influenced by aging, with the greater concentration in samples aged 45 and 55 d compared to samples aged 35 or 65 d postmortem. It should be noted that trends for volatile concentration due to postmortem aging could be partially attributed to the inherent variation between animal or carcass as each subprimal was assigned to a single postmortem aging period.

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