

Feeding microalgae meal (All-G Rich™; *Schizochytrium limacinum* CCAP 4087/2) to beef heifers. I: Effects on longissimus lumborum steak color and palatability^{1,2}

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ABSTRACT: The objective of this study was to examine effects of 4 levels of microalgae meal (All-G Rich, *Schizochytrium limacinum* CCAP 4087/2; Alltech Inc., Nicholasville, KY) supplementation to the diet of finishing heifers on longissimus lumborum (LL) steak PUFA content, beef palatability, and color stability. Crossbred heifers ($n = 288$; 452 ± 23 kg initial BW) were allocated to pens (36 pens and 8 heifers/pen), stratified by initial pen BW ($3,612 \pm 177$ kg), and randomly assigned within strata to 1 of 4 treatments: 0, 50, 100, and 150 g·heifer⁻¹·d⁻¹ of microalgae meal. After 89 d of feeding, cattle were harvested and LL were collected for determination of fatty acid composition and Warner–Bratzler shear force (WBSF), trained sensory panel evaluation, and 7-d retail color stability and lipid oxidation analyses. Feeding microalgae meal to heifers increased (quadratic, $P < 0.01$) the content of 22:6n-3 and increased (linear, $P < 0.01$) the content of 20:5n-3. Feeding increasing levels of microalgae meal did not impact total SFA or MUFA ($P > 0.25$) but tended ($P = 0.10$) to increase total PUFA in a quadratic manner ($P = 0.03$). Total omega-6 PUFA decreased (linear, $P = 0.01$) and total omega-3 PUFA

increased (quadratic, $P < 0.01$) as microalgae meal level increased in the diet, which caused a decrease (quadratic, $P < 0.01$) in the omega-6:omega-3 fatty acid ratio. Feeding microalgae meal did not affect WBSF values or sensory panel evaluation of tenderness, juiciness, or beef flavor scores ($P > 0.16$); however, off-flavor intensity increased with increasing concentration of microalgae meal in the diet (quadratic, $P < 0.01$). From d 5 through 7 of retail display, steaks from heifers fed microalgae meal had a reduced a* value and oxymyoglobin surface percentage, with simultaneous increased surface metmyoglobin formation (quadratic, $P < 0.01$). Lipid oxidation analysis indicated that at d 0 and 7 of display, as the concentration of microalgae meal increased in the diet, the level of oxidation increased (quadratic, $P < 0.01$). Muscle fiber type percentage or size was not influenced by the inclusion of microalgae meal in diets ($P > 0.19$); therefore, the negative effects of microalgae on color stability were not due to fiber metabolism differences. Feeding microalgae meal to finishing heifers improves PUFA content of beef within the LL, but there are adverse effects on flavor and color stability.

Key words: color, fatty acid, longissimus lumborum, microalgae, palatability

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INTRODUCTION

In a review of the role of meat in a healthy human diet, Givens et al. (2006) stated that as economies become more developed, the amount of animal-derived

foods increases. Omega-3 fatty acids are a family of PUFA that provide numerous health benefits, including reduced risks of cardiovascular disease, type 2 diabetes, and cancer (Ruxton et al., 2004; Calder, 2014). In particular, the long-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the most functionally active within the body (Calder, 2014). Both EPA and DHA are found in copious amounts in fatty fish such as salmon and trout but are relatively absent in beef products due to biohydrogenation of dietary PUFA in the rumen (Harfoot, 1978). Because most Americans do not

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consume adequate amounts of omega-3 PUFA (USDA and U.S. Department of Health and Human Services, 2010), research has focused on manipulating the fatty acid profile of beef as an alternative source of omega-3 fatty acids. The main strategy used to manipulate fatty acid profiles of beef has been through feeding oilseeds, plant oils, fish oil, marine algae, and fat supplements (Woods and Fearon, 2009). Supplementation of flaxseed and fish oil has increased the omega-3 content of beef derived from forage- and grain-fed cattle (Vatansever et al., 2000; Wistuba et al., 2006; Kronberg et al., 2011).

Increasing the omega-3 fatty acid content of beef may be appealing for some consumers, but polyunsaturated fats are susceptible to oxidation and, therefore, may cause adverse effects on meat quality. Increasing the omega-3 fatty acid content of beef has decreased color stability during display (LaBrune et al., 2008; Kronberg et al., 2011) and has increased the off-flavors of cooked product (Vatansever et al., 2000; Wistuba et al., 2006; LaBrune et al., 2008). Therefore, the objective of this study was to evaluate effects of feeding a microalgae meal during the finishing phase on LM fatty acid profiles and fresh meat color stability and palatability.

MATERIALS AND METHODS

All experimental procedures were approved by the Kansas State University Institutional Animal Care and Use Committee, and the Kansas State University Institutional Review Board approved procedures for the use of human subjects in sensory panel evaluations.

Heifer Management

Crossbred feedlot heifers (452 ± 23 kg initial BW) were housed in partially covered, concrete-surfaced pens (4.2 by 8.4 m; 36 pens and 8 heifers/pen) that provided 3.4 m of linear bunk space and were equipped with watering fountains between adjacent pens. Heifers were predominantly black hided (80%), consisting mostly of Black Angus and Black Angus crossbreds, with lesser numbers of gray- and red-hided heifers having phenotypes consistent with Charolais crossbreds and Red Angus. Prior to the start of the experiment, pens were blocked by initial pen BW ($3,612 \pm 177$ kg) and assigned within strata to 1 of 4 treatments. Treatments consisted of 0, 50, 100, and 150 g·heifer⁻¹·d⁻¹ of supplemental microalgae meal (All-G RichTM; *Schizochytrium limacinum* CCAP 4087/2; Alltech Inc., Nicholasville, KY). All treatment groups were fed a similar basal diet, but the feed additive premix for each treatment group was formulated to provide the appropriate amount of microalgae meal by substituting it for ground corn. Additionally, supplemental vitamin E was included in

all rations at 22 IU/kg of feed. Ractopamine hydrochloride (Optaflexx; Elanco Animal Health, Greenfield, IN) was supplemented for the final 28 d of the experiment at the rate of 400 mg·heifer⁻¹·d⁻¹. Heifers were fed once daily in fence line feed bunks that provided 28 linear centimeters of bunk space per animal. Daily rations were presented in quantities estimated to result in 227 g of unconsumed feed on the following day.

Loin Collection and Processing

At the completion of the 89-d feeding trial, a subset of black-hided heifers (527.5 ± 8.4 kg final BW; 3/pen) were randomly selected and transported to a commercial abattoir for harvest (Creekstone Farms, Arkansas City, KS). After a 48-h refrigeration period, strip loins (Institutional Beef Purchase Specifications number 180; NAMP, 2010) were removed from the left side of each carcass, vacuum packaged, and transported to the Kansas State University Meats Laboratory (Manhattan, KS) for processing. Twelve hours after arrival (72 h postmortem), two 1.27-cm thick steaks were removed from the anterior end of each loin for fatty acid and immunohistochemistry analyses. The remaining portion of each loin was weighed, vacuum packaged, and stored at $2 \pm 1^\circ\text{C}$ until 14 d postmortem.

Following 14 d of refrigerated storage, loins were reweighed and pH was measured at the geometric center of each loin using a meat pH meter (model HI 99163; Hanna Instruments, Smithfield, RI). Purge loss was calculated using the equation [(initial weight – final weight)/initial weight] × 100. Subsequently, four 2.54-cm thick steaks were fabricated from each loin, starting at the anterior end. Steak 1 was used for display d-0 lipid oxidation analysis and steak 2 was displayed under simulated retail conditions for 7 d before being used for lipid oxidation analysis. Steaks 3 and 4 were vacuum packaged and frozen at -40°C and subsequently used for Warner–Bratzler shear force (WBSF) and trained sensory panel analysis, respectively.

Fatty Acid Methyl Ester Analyses

Determination of fatty acid methyl esters (FAME) of ground beef samples was performed using a 1-step extraction/transesterification method as described by Sukhija and Palmquist (1988). Two hundred milligrams of freeze-dried ground beef was extracted and transesterified in methanol:benzene:acetyl chloride (20:27:3, vol/vol) and 2 mL of internal standard (methyl tridecanoate; 2.0 mg 13:0/mL of benzene). Samples were heated for 2 h at 70°C in a 70-mL sealed screw-capped tube. After cooling, 5 mL potassium carbonate and 2 mL benzene were added. The tubes were then vortexed and centri-

fused for 5 min at $500 \times g$ at 25°C to allow separation, and the FAME in the solvent were transferred to 2-mL vials. The FAME was analyzed using an Agilent Gas Chromatograph (model 7890A; Agilent Technologies, Inc., Santa Clara, CA). Separation of FAME was accomplished on a fused silica capillary column HP-88 (30 m by 0.25 mm by $0.20 \mu\text{m}$ [i.d.]; Agilent Technologies, Inc.), with hydrogen as the carrier gas (35 mL/min flow rate and 100:1 split ratio). The initial oven temperature was 80°C , which was held 1 min; then, the oven temperature was increased at $14^{\circ}\text{C}/\text{min}$ to 240°C and held 3 min. Injector and detector temperatures were at 280 and 300°C , respectively. Individual fatty acids were identified by comparing retention times using genuine external standard Supelco 37 (47885-U Supelco; Sigma-Aldrich, St. Louis, MO). Individual FAME were quantified as a percentage of total FAME analyzed.

Warner–Bratzler Shear Force and Sensory Analyses

Warner–Bratzler shear force and sensory analyses were conducted according to procedures outlined in the American Meat Science Association (AMSA) meat cookery and sensory guidelines (AMSA, 2015). Twenty-four hours prior to cooking, steaks were thawed on trays at $2.7 \pm 0.9^{\circ}\text{C}$. Before cooking, steaks were weighed and a thermocouple (30-gauge copper and constantan; Omega Engineering, Stamford, CT) was inserted into the geometric center of each steak. Steaks were cooked on clam-style grills (Cuisinart Griddler; Cuisinart, Stamford, CT) using the ribbed grill plate side set to a surface temperature of 232°C and removed from grills at 70°C . Following cooking, steaks were reweighed and the cook loss was determined using the equation [(initial weight – cooked weight)/initial weight] $\times 100$. After a 24-h chill period, six 1.27-cm cores were removed from each steak parallel to the muscle fiber and sheared once through the center using an Instron model 5569 testing machine (Instron, Canton, MA) with a Warner–Bratzler shear head attached (100 kg compression load cell and crosshead speed of 250 mm/min).

Sensory panel steaks were cooked according to procedures described for WBSF analyses. After cooking, steaks were cut into 1.27 by 1.27 by 2.54 cm pieces and presented to a 6- to 9-member trained sensory panel. Panelists were selected from a pool of 25 candidates from Kansas State University's Animal Sciences and Industry Department Manhattan, KS), and panelists were screened and trained according the AMSA meat cookery and sensory guidelines (AMSA, 2015). Selected panelists were oriented to strip loin steak evaluation procedures over 4 training sessions prior to initiation of panels by evaluating commodity beef steaks. Panelists were presented 2 pieces from each of 8 steaks ($n = 2$ per treatment) under

low intensity (<107.64 lumens) red incandescent lighting. Panelists evaluated samples for myofibrillar tenderness, juiciness, beef flavor intensity, connective tissue amount, overall tenderness, and off-flavor intensity using an 8-point scale (1 = extremely tough, extremely dry, extremely bland, abundant, extremely tough, or abundant, respectively, and 8 = extremely tender, extremely juicy, extremely intense, none, extremely tender, or none, respectively). A total of 12 separate panels were conducted to analyze all samples.

Simulated Retail Display

Steaks used for the 7-d simulated retail display were placed on 17S polystyrene foam trays with a Dri-Loc (Dri-Loc 50; Cryovac Sealed Air Corp., Duncan, SC) absorbent pad and overwrapped with polyvinyl chloride film (AEP Industries Inc., South Hackensack, NJ) with an oxygen transmission rate of $1,450 \text{ cm}^{-3} \cdot 645.2 \text{ cm}^{-2} \cdot 24 \text{ h}^{-1}$. Steaks were orientated on trays so the posterior end faced up and the medial portion of the steak was on the left side of the tray, as viewed from above. Steaks were displayed in coffin-style retail cases (model DMF 8; Tyler Refrigeration Corp., Niles, MI) under fluorescent lights (32 W Del-Warm White 3000° K; Philips Lighting Co., Somerset, NJ) that emitted a constant 24-h case average intensity of $2,230 \pm 34 \text{ lx}$. Case temperature was monitored using a Thermochron iButton (Maxim Integrated Products, Sunnyvale, CA). Average temperatures of the cases at steak package surfaces were $0.26 \pm 0.95^{\circ}\text{C}$ and the cases were defrosted twice daily (morning and evening) at 11°C for 30 min. Every 12 h, steaks were rotated in the cases from left to right and front to back to account for variation in temperature and light intensity within the cases. Readings for CIE L^* , a^* , and b^* and reflectance from 400 to 700 nm were taken at the 3 steak locations on each day of display using a HunterLab Miniscan EZ spectrophotometer (Illuminant A, 2.54-cm diameter aperture, 10° observer; Hunter Associates Laboratory, Reston, VA) every 24 h. Surface reflectance values at 473, 525, 572, and 700 nm were used to calculate surface percentages of metmyoglobin and oxymyoglobin using equations from Krzywicki (1979) as published in the AMSA *Meat Color Measurement Guidelines* (AMSA, 2012). Values from the 3 scans were used to calculate an average value for each steak.

Thiobarbituric Acid Reactive Substances

The extent of lipid oxidation during simulated retail display was assessed using the thiobarbituric acid reactive substances (TBARS) assay using procedures first described by Buege and Aust (1978) published in the AMSA color guidelines (AMSA, 2012). Briefly, steaks

were cut into 1.27 by 1.27 by 2.54 cm pieces, frozen in liquid nitrogen, and pulverized using a Waring blender (Waring Products Division, Hartford, CT). Duplicate 0.5-g subsamples of each steak were weighed into 15-mL conical tubes and stored at -80°C until analysis. Samples were heated in 2.5 mL of thiobarbituric acid stock solution (0.375% thiobarbituric acid, 15% trichloroacetic acid, and 0.25 *N* HCl) in a 100°C water bath for 10 min. Samples were cooled in room temperature water for 5 min and centrifuged at 4°C and $5,000 \times g$ for 10 min. One milliliter of the resulting supernatant was transferred to a cuvette and absorbance was read at 532 nm (Eon Microplate Spectrophotometer; BioTek Instruments, Inc., Winooski, VT). Values were expressed as milligrams malonaldehyde/kilogram of muscle calculated using the equation published in the AMSA *Meat Color Measurement Guidelines* (AMSA, 2012).

Immunohistochemistry

Analyses of muscle fiber characteristics were conducted following procedures described by Phelps et al. (2014). Briefly, one 1 by 1 by 1.27 cm sample was collected from the geometric center of the medial, mid-lateral, and lateral portions of the longissimus lumborum (**LL**; $n = 3/\text{steak}$). Five-micrometer cryosections were cut using a Microm 550 cryostat (Thermo Fisher Scientific Inc., Waltham, MA). Cryosections were blocked using 10% horse serum in PBS. Afterwards, cryosections were incubated in the following primary antibodies diluted in blocking solution for 1 h: anti-dystrophin (Thermo Fisher Scientific Inc.), anti-slow myosin heavy chain (BA-D5; Developmental Studies Hybridoma Bank, Iowa City, IA), and anti-myosin heavy chain all but IIX (BF-35; Developmental Studies Hybridoma Bank). Following a wash step, cryosections were incubated in secondary antibodies (AlexaFluor 594, 633, and 488 for anti-dystrophin, BA-D5, and BF-35, respectively; Thermo Fisher Scientific Inc.) in blocking solution for 45 min. Cryosections were cover slipped and photomicrographs were captured using a Nikon Eclipse TI-U inverted microscope with an attached DS-QiMC digital camera at a 100x magnification (Nikon Instruments Inc., Melville, NY).

For each steak location, a minimum of 500 fibers were analyzed for myosin heavy chain type and muscle fiber cross-sectional area (**CSA**) using NIS-Elements software (Basic Research 3.3; Nikon Instruments, Inc., Lewisville, TX). The area constrained by α -dystrophin immunostaining defined individual fibers for CSA measurements. Fibers that stained positive for the BA-D5 antibody were classified as type I fibers. Fibers that stained positive for BF-35 but that were negative for BA-D5 were classified as type IIA fibers. All fibers that were negative

for BF-35 were classified as type IIX fibers (Schiaffino et al., 1989; Moreno-Sánchez et al., 2008).

Statistical Analyses

Data were analyzed as a randomized complete block design using the PROC MIXED procedure of SAS 9.4 (SAS Inst. Inc, Cary, NC) with pen as the experimental unit and animal as the observational unit. Treatment was the fixed effect and initial BW block was the random effect. For retail shelf life data, data were analyzed as a randomized complete block design with repeated measures. Day of display served as the repeated measure with steak (observational unit) as the subject and compound symmetry as the covariance structure. Preplanned linear and quadratic contrasts were tested for all data and within each day of display for the color data. Differences were considered significant at $P \leq 0.05$ and regarded as tendencies at $0.05 > P \leq 0.10$.

RESULTS

Longissimus Lumborum Fatty Acid Content

Fatty acid profiles of LL steaks from the 4 treatments are presented in Table 1. As the amount of microalgae meal in the diet increased, the amount of 18:1 *trans*-11 increased (quadratic, $P < 0.01$) and the amount of 18:2 n -6 *cis* and 20:3 n -6 decreased (linear, $P < 0.01$). As the amount of microalgae meal in the diet increased, the content of CLA *cis*-9, *trans*-11 increased (quadratic, $P < 0.01$). Also, as the amount of microalgae meal in the diet increased, the amount of 24:1 decreased (quadratic, $P < 0.01$). The amount of 20:5 n -3 and 22:6 n -3 increased at a greater rate (quadratic, $P < 0.01$) as the microalgae meal content of the diet was increased. Feeding microalgae meal affected ($P = 0.01$) 22:5 n -3 content, but no linear or quadratic responses were found ($P > 0.19$). Feeding increasing levels of microalgae meal did not impact total SFA or MUFA ($P > 0.25$) but tended ($P = 0.10$) to increase total PUFA in a quadratic manner ($P = 0.03$). Total omega-6 PUFA decreased (linear, $P = 0.01$) and total omega-3 PUFA increased (quadratic, $P < 0.01$) as microalgae meal level increased in the diet, which caused a decrease (quadratic, $P < 0.01$) in the omega-6:omega-3 fatty acid ratio.

Warner-Bratzler Shear Force and Sensory Analyses

There were no treatment effects on ultimate pH or purge loss from loins stored 14 d postmortem ($P > 0.20$; Table 2). Increasing microalgae meal in the diet did not affect cook loss or WBSF ($P > 0.15$). Trained sensory panelists detected no differences among

Table 1. Least squares means of fatty acid profiles of longissimus lumborum steaks from heifers fed 0, 50, 100, or 150 g·heifer⁻¹·d⁻¹ of microalgae meal (All-G RichTM, *Schizochytrium limacinum* CCAP 4087/2, Alltech Inc., Nicholasville, KY)

Fatty acid methyl ester ¹	Algae, g·heifer ⁻¹ ·d ⁻¹				SEM	P-value ²		
	0	50	100	150		Algae	Linear	Quadratic
14:0	1.79	1.94	1.66	1.98	0.18	0.51	0.62	0.15
14:1	0.48	0.55	0.44	0.56	0.06	0.51	0.46	0.19
15:0	0.25	0.25	0.22	0.27	0.02	0.40	0.32	0.22
15:1	0.04	0.04	0.04	0.04	0.01	0.14	0.04	0.54
16:0	16.13	17.51	14.72	17.79	1.40	0.33	0.50	0.09
16:1	2.09	2.29	1.77	2.13	0.20	0.25	0.16	0.12
17:0	0.69	0.69	0.59	0.75	0.07	0.31	0.34	0.20
17:1	0.56	0.55	0.45	0.56	0.04	0.22	0.09	0.21
18:0	7.87	8.01	6.61	7.68	0.64	0.38	0.13	0.31
18:1 <i>cis</i> -9	22.57	24.00	18.60	22.42	1.96	0.22	0.10	0.15
18:1 <i>trans</i> -11	1.74	2.44	2.50	3.61	0.26	<0.01	<0.01	<0.01
18:1 <i>cis</i> -11	0.86	0.93	0.75	0.88	0.06	0.22	0.16	0.10
18:2 <i>n</i> -6 <i>cis</i>	2.36	2.34	1.96	2.24	0.12	0.03	0.01	0.20
18:2 <i>n</i> -6 <i>trans</i>	0.04	0.04	0.04	0.05	0.01	0.14	0.64	0.13
18:3 <i>n</i> -6 <i>cis</i>	0.02	0.01	0.02	0.02	0.001	<0.01	0.99	0.05
18:3 <i>n</i> -3	0.19	0.20	0.15	0.19	0.02	0.13	0.09	0.08
20:0	0.04	0.04	0.03	0.04	0.01	0.31	0.08	0.40
20:1	0.09	0.10	0.07	0.09	0.01	0.29	0.11	0.32
20:2	0.07	0.07	0.07	0.08	0.01	0.27	0.84	0.06
20:3 <i>n</i> -6	0.15	0.13	0.13	0.13	0.01	<0.01	<0.01	0.10
20:4 <i>n</i> -6 and 22:1 <i>n</i> -9 ³	0.41	0.41	0.41	0.41	0.01	0.92	0.93	0.48
20:5 <i>n</i> -3	0.05	0.09	0.17	0.22	0.01	<0.01	<0.01	<0.01
22:5 <i>n</i> -3	0.12	0.10	0.12	0.12	0.01	0.01	0.19	0.34
22:6 <i>n</i> -3	0.02	0.13	0.19	0.19	0.01	<0.01	<0.01	<0.01
23:0	0.02	0.02	0.02	0.02	0.01	0.72	0.90	0.26
24:0	0.04	0.04	0.04	0.05	0.01	0.25	0.98	0.22
24:1	0.04	0.03	0.02	0.02	0.01	<0.01	<0.01	<0.01
CLA <i>cis</i> -9, <i>trans</i> -11	0.16	0.21	0.17	0.23	0.02	0.07	0.72	0.01
CLA <i>trans</i> -10, <i>cis</i> -12	0.02	0.02	0.02	0.02	0.01	0.35	0.51	0.12
CLA <i>trans</i> -9, <i>trans</i> -11	0.07	0.09	0.08	0.11	0.01	0.01	0.18	0.02
Total SFA ⁴	26.84	28.51	23.88	28.56	2.27	0.39	0.35	0.13
Total MUFA ⁵	28.48	30.94	24.65	30.25	2.53	0.25	0.25	0.09
Total PUFA ⁶	3.20	3.37	3.02	3.53	0.17	0.10	0.59	0.03
Total omega-6 PUFA ⁷	2.57	2.53	2.13	2.44	0.12	0.03	0.01	0.23
Total omega-3 PUFA ⁸	0.37	0.52	0.62	0.73	0.02	<0.01	<0.01	<0.01
PUFA:SFA ratio ⁹	0.13	0.13	0.14	0.13	0.01	0.40	0.10	0.56
Omega-6:omega-3 ratio ¹⁰	7.02	4.84	3.42	3.37	0.18	<0.01	<0.01	<0.01
Total fatty acids	59.09	63.41	52.13	62.94	4.91	0.30	0.30	0.10

¹Milligrams per gram wet tissue.

²Probability values for overall *F*-test as well as contrasts for linear and quadratic effects of algae.

³Fatty acids 20:4*n*-6 and 22:1*n*-9 eluted together.

⁴Total SFA = 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 24:0.

⁵Total MUFA = 14:1 + 15:1 + 16:1 + 18:1 *cis*-9 + 18:1 *cis*-11 + 18:1 *trans*-11 + 20:1 + 24:1.

⁶Total PUFA = 18:2*n*-6 *cis* + 18:2*n*-6 *trans* + 18:3*n*-6 *cis* + 18:3*n*-3 + 20:2 + 20:3*n*-6 + 20:4*n*-6 and 22:1*n*-9 + 20:5*n*-3 + 22:5*n*-3 + 22:6*n*-3 + CLA *cis*-9, *trans*-11 + CLA *trans*-10, *cis*-12 + CLA *trans*-9, *trans*-11.

⁷Total omega-6 PUFA = 18:2*n*-6 *cis* + 18:2*n*-6 *trans* + 18:3*n*-6 *cis*.

⁸Total omega-3 PUFA = 18:3*n*-3 + 20:5*n*-3 + 22:5*n*-3 + 22:6*n*-3.

⁹PUFA:SFA = (18:2*n*-6 *cis* + 18:2*n*-6 *trans* + 18:3*n*-6 *cis* + 18:3*n*-3 + 20:2 + 20:3*n*-6 + 20:4*n*-6 and 22:1*n*-9 + 20:5*n*-3 + 22:5*n*-3 + 22:6*n*-3 + CLA *cis*-9, *trans*-11 + CLA *trans*-10, *cis*-12 + CLA *trans*-9, *trans*-11)/(14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 23:0 + 24:0).

¹⁰*n*-6:*n*-3: (18:2*n*-6 *cis* + 18:2*n*-6 *trans* + 18:3*n*-6 *cis*)/(18:3*n*-3 + 20:5*n*-3 + 22:5*n*-3 + 22:6*n*-3).

Table 2. Least squares means of objective and subjective cooked meat attributes of longissimus lumborum steaks from heifers fed 0, 50, 100, or 150 g·heifer⁻¹·d⁻¹ of microalgae meal (All-G RichTM, *Schizochytrium limacinum* CCAP 4087/2, Alltech Inc., Nicholasville, KY)

Item	Algae, g·heifer ⁻¹ ·d ⁻¹				SEM	P-value ¹		
	0	50	100	150		Treatment	Linear	Quadratic
Objective measures								
Ultimate pH ²	5.65	5.62	5.62	5.64	0.03	0.20	0.27	0.77
Purge loss, ³ %	1.48	1.30	1.19	1.16	0.15	0.34	0.11	0.46
Cook loss, ⁴ %	23.08	23.68	22.22	22.85	0.47	0.15	0.09	0.16
WBSF, ⁵ kg	3.48	3.47	3.63	3.35	0.16	0.65	0.63	0.36
Trained sensory panel measures ⁶								
Myofibrillar tenderness	5.85	5.92	5.77	5.92	0.12	0.79	0.67	0.35
Juiciness	4.99	5.16	5.08	5.17	0.10	0.49	0.48	0.18
Beef flavor intensity	5.07	5.08	5.11	4.93	0.06	0.16	0.58	0.34
Connective tissue	6.71	6.61	6.51	6.63	0.10	0.50	0.17	0.89
Overall tenderness	5.96	5.97	5.83	6.03	0.12	0.64	0.50	0.38
Off-flavor intensity	7.55	7.48	7.19	6.66	0.10	<0.01	<0.01	<0.01

¹Probability values for overall *F*-test as well as contrasts for linear and quadratic effects of algae.

²Ultimate pH was recorded at d 14 postmortem at the geometric center of each loin.

³[(initial weight – stored weight)/initial weight] × 100.

⁴[(precooked weight – cooked weight)/precooked weight] × 100.

⁵WBSF = Warner–Bratzler shear force.

⁶Myofibrillar tenderness: 1 = extremely tough and 8 = extremely tender; juiciness: 1 = extremely dry and 8 = extremely juicy; beef flavor intensity: 1 = extremely bland and 8 = extremely intense; connective tissue amount: 1 = abundant and 8 = none; overall tenderness: 1 = extremely tough and 8 = extremely tender; off-flavor intensity: 1 = abundant and 8 = none.

treatments for myofibrillar tenderness, juiciness, beef flavor intensity, connective tissue amount, or overall tenderness ($P > 0.16$). Off-flavor intensity increased (quadratic, $P < 0.01$) as the microalgae meal concentration of the diet increased.

Simulated Retail Display

As expected, day of display impacted all color measurements in a manner consistent with color deterioration during retail display ($P < 0.01$). Additionally, there were treatment × day interactions for all color attributes and TBARS values ($P < 0.01$). Therefore, within each day of display, linear and quadratic contrasts of microalgae meal treatments were analyzed. From the outset of the display period through d 2 there was a decrease in L^* value as the concentration of microalgae meal in the diet was increased (linear, $P < 0.03$; Fig. 1). This was also seen on d 4 of display (linear, $P < 0.01$). On d 3, 5, and 6 of display, the magnitude of the decrease in L^* due to increasing the microalgae meal content of the diet tended to decline (quadratic, $P < 0.07$) and continued to decline through d 7 of display (quadratic, $P = 0.05$).

In contrast to the L^* value, there was no effect of treatment on a^* and b^* values on d 0 of display ($P > 0.38$; Fig. 1); however, on d 1, treatment affected ($P < 0.03$) a^* and b^* values of steaks. On d 2, 3, and 4 of display, as the content of microalgae meal was increased in the diet, there were decreases in a^* and b^* values (linear, $P <$

0.04). On d 5, 6, and 7, the magnitude of decreases in a^* and b^* values increased as the concentration of microalgae meal in the diet increased (quadratic, $P < 0.03$).

The discoloration patterns observed for a^* and b^* measurements were also seen for steak surface oxymyoglobin and metmyoglobin accumulation (Fig. 2). From d 0 through 4 of display, increasing the amount of microalgae meal in the diet increased (linear, $P = 0.04$; quadratic, $P = 0.06$) the percentage of surface metmyoglobin. On d 0 of display, increasing microalgae meal in the diet decreased (linear, $P < 0.01$) the percentage of surface oxymyoglobin, but from d 1 to 2, treatment only tended to affect the surface oxymyoglobin percentage ($P < 0.06$). During d 3 and 4 of display, the surface oxymyoglobin percentage decreased as microalgae meal in the diet increased (linear, $P < 0.01$) whereas the surface metmyoglobin percentage linearly increased ($P < 0.01$) on d 3 and tended to increase in a quadratic fashion ($P = 0.08$) on d 4. By d 5 and through d 7 of the display period, the decrease in the surface oxymyoglobin percentage and subsequent increase in the metmyoglobin percentage became more severe as the concentration of microalgae meal in the diet was elevated (quadratic, $P < 0.04$).

Thiobarbituric Acid Reactive Substances

The extent of lipid oxidation during retail display was measured on d 0 and 7 of display (Fig. 3). There was a treatment × day interaction ($P < 0.01$); therefore,

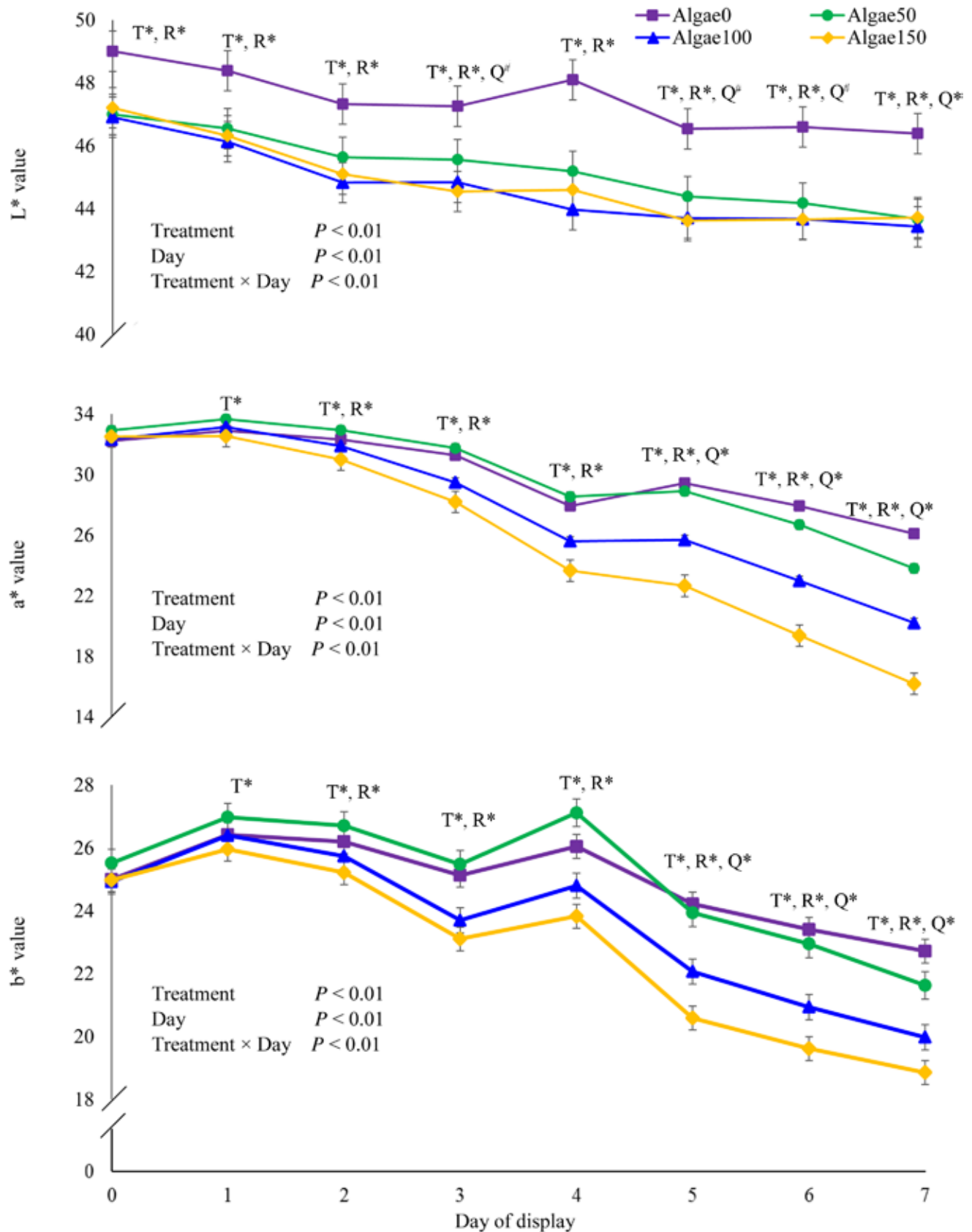


Figure 1. Longissimus lumborum steak L* (lightness; 0 = black and 100 = white), a* (redness; -60 = green and 60 = red), and b* (blueness; -60 = blue and 60 = yellow) values from heifers supplemented 0, 50, 100, and 150 g·heifer⁻¹·d⁻¹ of microalgae meal (Algae0, Algae50, Algae100, and Algae150, respectively; All-G Rich™, *Schizochytrium limacinum* CCAP 4087/2; Alltech Inc., Nicholasville, KY). Steaks were displayed under simulated retail conditions for 7 d. T = treatment effect; R = linear effect of algae; Q = quadratic effect of algae; *significant effect ($P \leq 0.05$); #tendency ($P \leq 0.10$).

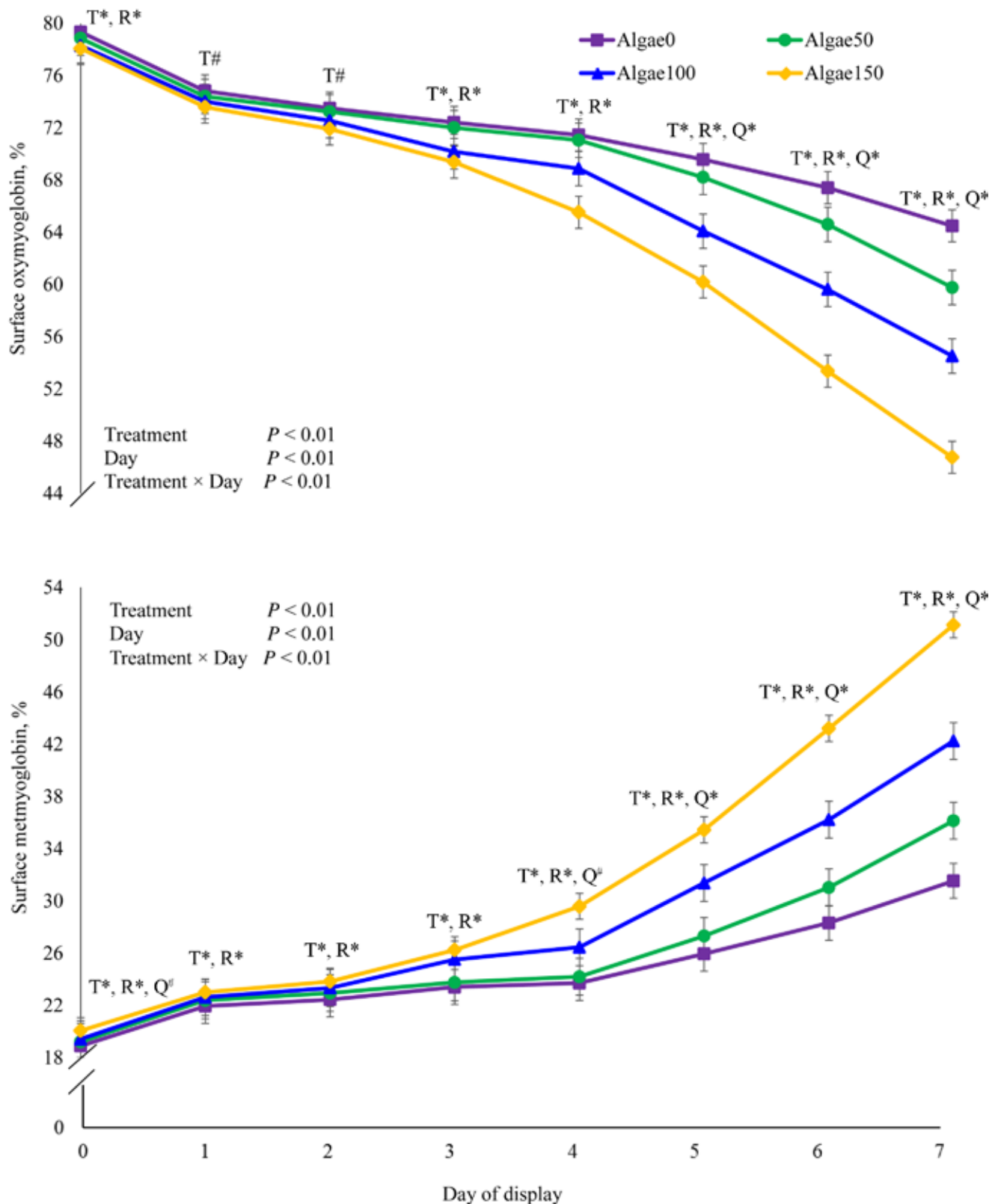


Figure 2. Longissimus lumborum steak surface oxymyoglobin and metmyoglobin percentage from heifers supplemented 0, 50, 100, and 150 g·heifer⁻¹·d⁻¹ of microalgae meal (Algae0, Algae50, Algae100, and Algae150, respectively; All-G Rich™, *Schizochytrium limacinum* CCAP 4087/2; Alltech Inc., Nicholasville, KY). Steaks were displayed under simulated retail conditions for 7 d and percent oxymyoglobin and metmyoglobin were calculated using the equations of Krzywicki (1979). T = treatment effect; R = linear effect of algae; Q = quadratic effect of algae; *significant effect ($P \leq 0.05$); #tendency ($P \leq 0.10$).

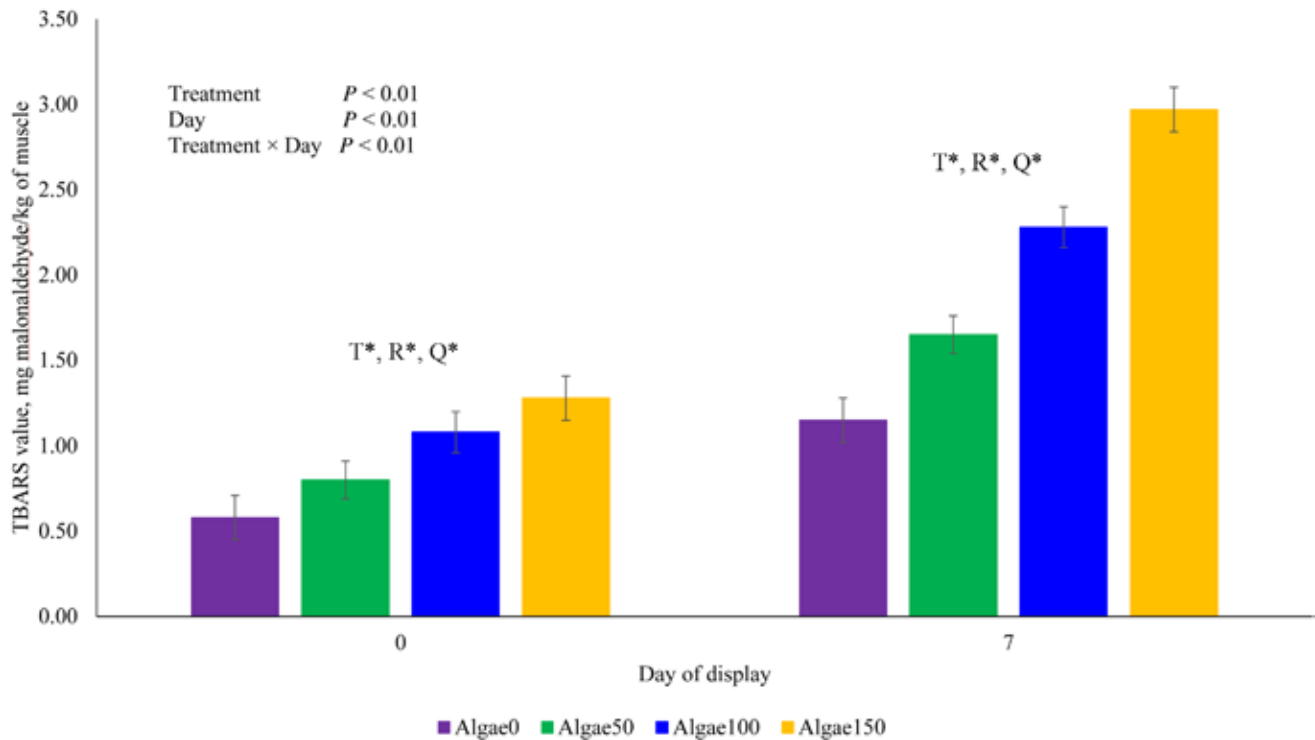


Figure 3. Day 0 and 7 longissimus lumborum steak lipid oxidation from heifers supplemented 0, 50, 100, and 150 g·heifer⁻¹·d⁻¹ of microalgae meal (Algae0, Algae50, Algae100, and Algae150, respectively; All-G Rich™, *Schizochytrium limacinum* CCAP 4087/2; Alltech Inc., Nicholasville, KY). Thiobarbituric acid reactive substances (TBARS) assay was conducted according to procedures outlined in the American Meat Science Association color guidelines (AMSA, 2012). Values were expressed as a TBARS expressed in milligrams malonaldehyde/kilogram of muscle. T = treatment effect; R = linear effect of algae; Q = quadratic effect of algae; *significant effect ($P \leq 0.05$).

contrasts were conducted within each day of display. On both days of display, feeding increasing concentrations of microalgae meal in the diet increased lipid oxidation (quadratic, $P < 0.01$).

Immunohistochemistry

There were no treatment effects on the distribution of type I, IIA, and IIX fibers within the LL ($P > 0.30$; Fig. 4). Additionally, there were no treatment effects on the CSA of each fiber type within the LL ($P > 0.16$; Table 3).

DISCUSSION

In the United States, meat contributes more than 40% of daily protein intake and 20% of daily fat intake (Daniel et al., 2011). Beef is one major meat protein source in the United States but is regarded as having relatively high concentrations of SFA compared with other protein sources. The intake of fat from meat has been a public health discussion for over 60 yr following the first recommendations of the American Heart Association to reduce intake of dietary cholesterol, saturated fat, and total fat to prevent cardiovascular disease (Eckel et al., 2013). According to the 2015 *Dietary Guidelines for Americans*, 20 to 35% of a person's daily calories should originate from fat but less than 10% should be from saturated fats

and the remainder from unsaturated fats (U.S. Department of Health and Human Services and USDA, 2015). Due to the undesirable fatty acid profile of beef and changes in dietary recommendations over time, researchers have explored nutritional regimens that increase the PUFA content of meat. Although feeding microalgae meal only tended to increase total PUFA, it reduced the amount of omega-6 PUFA and increased the amount of omega-3 PUFA, leading to a reduction in the omega-6:omega-3 ratio. The reduction of the omega-6:omega-3 ratio from 7:1 for steaks from heifers fed 0 g of microalgae/d (Algae0) to 3.4:1 for steaks from heifers fed 100 g of microalgae/d (Algae100) and 150 g of microalgae/d (Algae150) meets dietary recommendations for consuming foods with an omega-6:omega-3 ratio below a 4:1 for health benefits (Simopoulos, 2002). In other studies, Mandell et al. (1997) reported that feeding steers a diet containing 10% fish meal for 168 d reduced the omega-6:omega-3 ratio from 5.6 to 1.72 and Dunne et al. (2011) reported that supplementing heifers up to 275 g of rumen-protected fish oil reduced the ratio from 4.3 to 2.04.

Givens et al. (2000) concluded that fish oil/meal studies demonstrate the greatest ability to increase the DHA and EPA content of beef; however, questions about maintaining sustainable sources of fish oil/meal that are consistent in quality remain. Therefore, Givens et al. (2000) identified microalgae as an alternative to

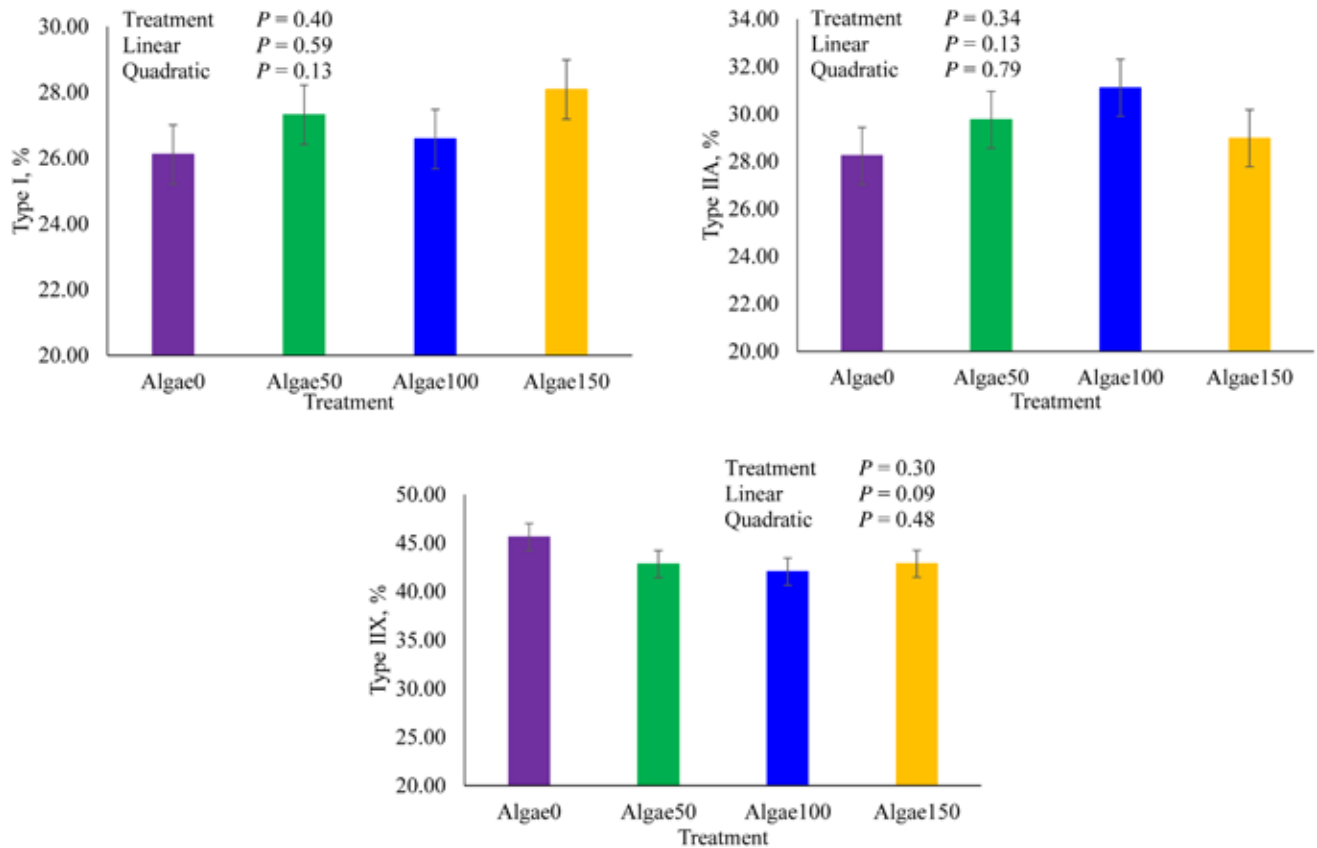


Figure 4. Muscle fiber type distribution of the longissimus lumborum from heifers supplemented 0, 50, 100, and 150 g·heifer⁻¹·d⁻¹ of microalgae meal (Algae0, Algae50, Algae100, and Algae150, respectively; All-G RichTM, *Schizochytrium limacinum* CCAP 4087/2; Alltech Inc., Nicholasville, KY). The methods of Phelps et al. (2014) were followed for immunostaining. Fibers that stained positive for the BA-D5 (Developmental Studies Hybridoma Bank, Iowa City, IA) antibody were categorized as type I fibers. Fibers that stained positive for BF-35 (Developmental Studies Hybridoma Bank) but were negative for BA-D5 were categorized as type IIA fibers. All fibers that were negative for the BF-35 antibody were categorized as type IIX fibers (Schiaffino et al., 1989; Moreno-Sánchez et al., 2008).

fish oil/meal. In the current study, as the microalgae content of the diet of heifers increased, EPA and DHA content of the LL increased. Compared with steaks from heifers fed no microalgae meal in the diet, steaks from heifers fed 150 g·heifer⁻¹·d⁻¹ of microalgae meal had 850 and 340% greater DHA and EPA concentrations, respectively. The DHA finding is in agreement with Franklin et al. (1999), who found a similar increase in the DHA content of milk from cows fed 910 g of protected microalgae. When compared with literature on fish oil, elevation of DHA and EPA in the current study are greater than increases reported by Vatanserver et al. (2000), Scollan et al. (2001), and Wistuba et al. (2007); however, Mandell et al. (1997) reported similar increases in DHA and EPA when fish meal was included at 10% of the diet. Dunne et al. (2011) found that increasing the content of ruminally protected fish oil produced the same quadratic increase in DHA content of neutral lipids but saw no response for EPA in neutral lipids.

Although there are few studies documenting the impact of microalgae on beef fatty acid profiles, there are 2 studies that use lamb models. Cooper et al. (2004) reported that adding microalgae to diets also containing fish oil

increased lamb LM EPA and DHA phospholipids by 127 and 39%, respectively, compared with diets containing linseed oil. Also, Cooper et al. (2004) reported that adding microalgae to diets containing protected lipid supplement increased lamb LM EPA and DHA content of phospholipids by 329 and 377%, respectively, compared with diets containing linseed oil. In the neutral lipid fraction, including microalgae in diets containing fish oil increased EPA and DHA by 125 and 575%, respectively, compared with diets containing linseed oil. Additionally, including microalgae in diets containing a protected lipid supplement substantially increased EPA and DHA in the neutral lipid fraction compared with diets containing linseed oil. Using the same treatments as Cooper et al. (2004), Nute et al. (2007) reported that adding microalgae to diets containing fish oil increased EPA and DHA by 127 and 25%, respectively, in the phospholipid fraction compared with diets containing linseed oil. Also, Nute et al. (2007) reported that adding microalgae to diets containing a protected lipid supplement increased EPA and DHA by 373 and 377%, respectively, in the phospholipid fraction compared with diets containing linseed oil. Therefore, these findings would indicate that microalgae may serve

Table 3. Least squares means of longissimus lumborum type I, IIA, and IIX muscle fiber cross-sectional area from heifers fed 0, 50, 100, or 150 g·heifer⁻¹·d⁻¹ of microalgae meal (All-G RichTM, *Schizochytrium limacinum* CCAP 4087/2, Alltech Inc., Nicholasville, KY)

Cross-sectional area, μm^2	Algae, g·heifer ⁻¹ ·d ⁻¹				SEM	P-value ¹		
	0	50	100	150		Treatment	Linear	Quadratic
Type I	2,601	2,321	2,289	2,521	119	0.19	0.17	0.84
Type IIA	2,794	2,692	2,710	2,676	139	0.93	0.67	0.62
Type IIX	4,171	3,809	3,954	4,020	181	0.56	0.59	0.41

¹Probability values for overall *F*-test as well as contrasts for linear and quadratic effects of algae.

as a better source of dietary DHA and EPA for ruminant animals than fish oil or fish meal.

The results of fish oil and microalgae studies are encouraging for improving the fatty acid profile of animal products, but some of the same studies indicate there may be negative effects on both palatability and shelf life. Tenderness and flavor are the 2 most important attributes consumers evaluate when determining the quality of their eating experience (Beermann, 2009; O'Quinn et al., 2012). In the current study, feeding microalgae did not influence measures of objective and subjective tenderness. Because muscle fiber CSA can influence cooked meat tenderness (Crouse et al., 1991; Ebarb et al., 2016), the absence of an effect of microalgae feeding on CSA helps explain the lack of differences in tenderness. In agreement, Nute et al. (2007) reported that sensory panel tenderness and juiciness scores were not influenced by feeding microalgae to lambs. Wistuba et al. (2006) reported that supplementing steers 3% fish oil during the finishing phase did not affect WBSF or steak cook loss. Using 2 separate sensory panels, Vatansever et al. (2000) found that sirloin steaks from steers fed fish oil had slightly poorer tenderness scores when panelists evaluated the steaks on an 8-point scale but there were no differences when panelists used a 100-mm line scale.

The adverse impact of microalgae feeding on sensory panel off-flavor ratings of LL steaks could be a concern for retailers. When examining off-flavor descriptors recorded by panelists in this study, oxidized, grassy, and fishy were the 3 most commonly recorded off-flavors. An increase in off-flavor intensity of products with increased levels of omega-3 fatty acids is commonly reported because these fatty acids are more susceptible to lipid oxidation (Jacobsen, 2008). In contrast to the present study but analyzing a different animal product, Franklin et al. (1999) found that microalgae did not alter the flavor of milk. Vatansever et al. (2000) reported that supplementing fish oil to steers increased fishy and rancid flavors and increased the intensity of abnormal flavors in longissimus dorsi steaks. Similarly, Wistuba et al. (2006) reported that supplementing fish oil elevated fishy flavors in LL steaks. In lambs, sensory panelists rated chops from microalgae meal-supplemented

lambs as having the most "rancid" off-flavors (Nute et al., 2007). These chops had the greatest proportion of DHA in the muscle, which correlated with reductions in lamb flavor and increases in abnormal lamb, rancid, and fishy flavors. Additionally, muscle EPA content correlated to fishy off-flavors more than DHA content. Nute et al. (2007) concluded that the rancid flavors detected by panelists were due to the greater PUFA content of the microalgae meal and fish oil supplemented meat, which possessed more oxidation-susceptible double bonds.

Color is the most important attribute that consumers evaluate when making purchasing decisions (Mancini and Hunt, 2005). Faustman et al. (1998) reported that consumers prefer bright, cherry red-colored steaks; therefore, maintaining the redness of steaks during display will help keep products available for purchase longer. In the current study, differences in the color of steaks from each treatment group are depicted in Fig. 5. All measures of surface color followed the typical patterns associated with steak discoloration; however, steaks of microalgae-fed heifers had accelerated discoloration in all measures. This is especially true for the accelerated decreases in surface oxymyoglobin percentage and *a** and the simultaneous increase in metmyoglobin percentage as concentration of microalgae fed and display time were increased. In agreement, color deteriorated quicker during the final 4 d of an 11-d retail display study when lambs were fed a fish oil/microalgae-supplemented diet (Nute et al., 2007). In minced beef models, feeding fish oil adversely affected color saturation (Vatansever et al., 2000) and surface metmyoglobin formation (Daly et al., 2007). Vatansever et al. (2000) also demonstrated the accelerated increase in metmyoglobin formation with fish oil inclusion compared with other treatments as display time increased. In contrast to these studies, minced neck muscle from heifers supplemented ruminally protected fish oil did not exhibit differences in *L**, *a**, and oxymyoglobin and metmyoglobin formation (Dunne et al., 2011). The authors hypothesized that the difference in their results compared with other studies were due the neck muscle being used and muscles from this from this area having muscle fibers that are more oxidative and smaller, which leads to greater antioxidant capability.

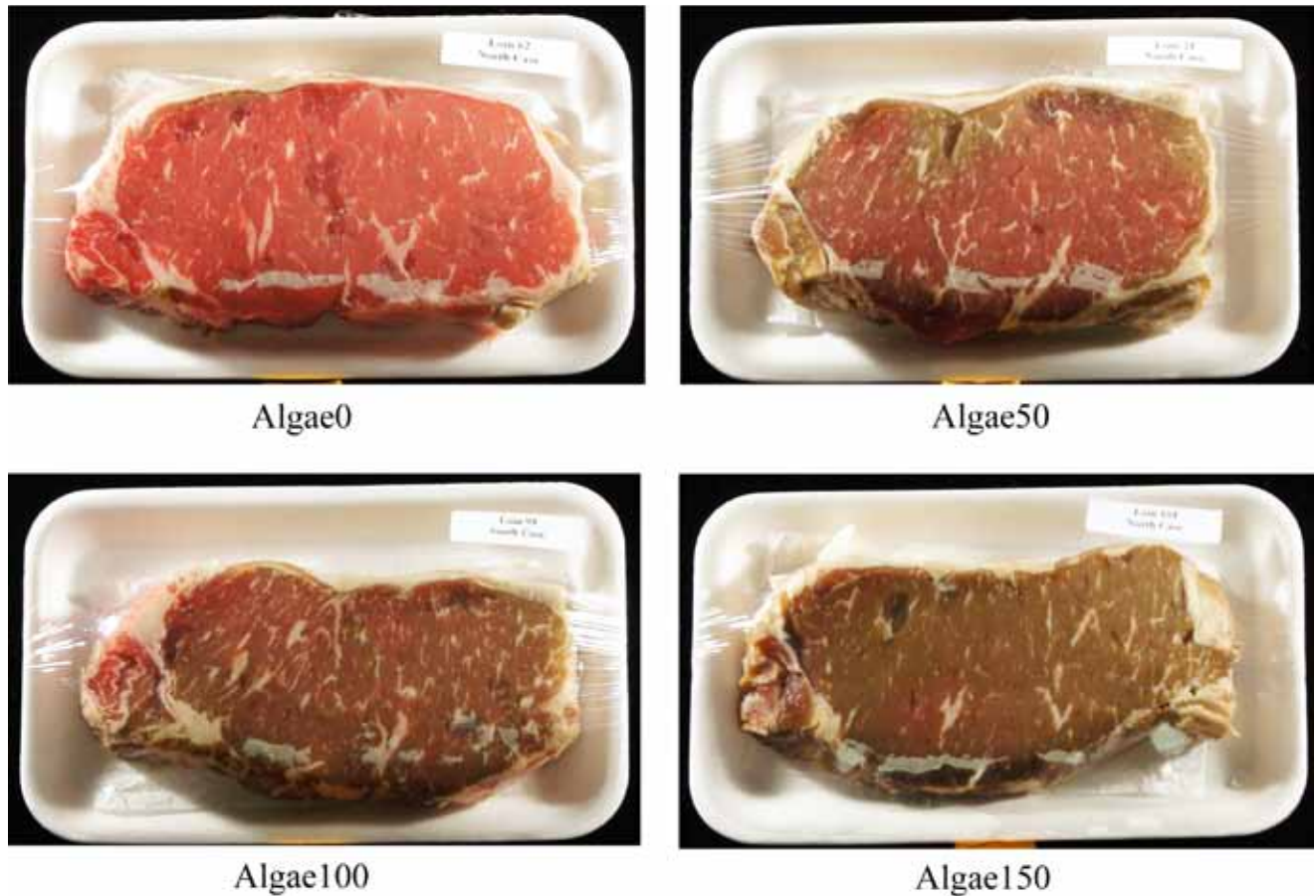


Figure 5. Day-7 photographs of representative steaks from heifers supplemented 0, 50, 100, and 150 g·heifer⁻¹·d⁻¹ of microalgae meal (Algae0, Algae50, Algae100, and Algae150, respectively; All-G Rich™, *Schizochytrium limacinum* CCAP 4087/2; Alltech Inc., Nicholasville, KY). Steaks were placed on 17S Styrofoam trays (Dri-Loc 50; Cryovac Sealed Air Corp., Duncan, SC), overwrapped with polyvinyl chloride film (AEP Industries, South Hackensack, NJ) with an oxygen transmission rate of 1,450 cm³·645.2 cm⁻²·24 h⁻¹, and displayed under simulated retail conditions for 7 d.

A muscle's fiber composition has an effect on its postmortem metabolic properties that impact color such as oxygen consumption rate and metmyoglobin reducing ability (Mancini and Hunt, 2005). Because muscle fiber type percentage was not altered by microalgae inclusion, a change in the metabolism of the muscle is not responsible for the rapid color deterioration demonstrated in the current study. Therefore, the color deterioration observed in the current study is likely due to the shift to a more unsaturated fatty acid profile and increased oxidation of these fatty acids. Beef products with greater PUFA content are susceptible to oxidation during display (Yang et al., 2002). At both the beginning and end of display, lipid oxidation as measured by TBARS increased with increasing microalgae meal concentrations of the diet. Also of importance is the amount of oxidation detected on d 0 of display in relation to the flavor problems noted above. Younathan and Watts (1959) stated that a TBARS value of 1 indicated a level of lipid oxidation at which consumers detected rancidity. On d 0 of display, which corresponds to d 14 of aging, both the Algae100 and Algae150 treatments displayed TBARS values greater than 1. Because lipids

having more unsaturated fatty acids are more susceptible to oxidation, they can have greater TBARS values (Jacobsen, 2008). Because Algae100 and Algae150 treatments had greater unsaturated fatty acid content, this may indicate why sensory panelists detected more off-flavors in steaks from these treatments. Vatanserver et al. (2000) reported that steaks from steers fed fish oil had increased TBARS values on d 4, 8, and 11 of display. Additionally, steaks from fish oil-supplemented steers had values over 1 on d 8 and 11 of display, whereas the other treatments never achieved this value. Nute et al. (2007) reported that chops from lambs supplemented fish oil/microalgae had greater TBARS value than those from lambs supplemented linseed oil and protected lipid supplement. Interestingly, the fish oil/microalgae steaks also had reduced vitamin E content than these 2 treatments. Dunne et al. (2011) also found in their dose titration study that the greatest level of fish oil supplemented caused minced beef to have the greatest TBARS value at d 10 of display; however, there were no differences in surface color properties. The authors speculated that their muscle had a high α -tocopherol content that inhibited treatment differences. Feeding

elevated levels of vitamin E during the finishing phase can help mitigate lipid oxidation and color deterioration during display (Yang et al., 2002; Gobert et al., 2010). In the current study, the antioxidant content of the diet was not adjusted to account for potential increases in PUFA due to the inclusion of microalgae. Future studies using this microalgae product should focus on altering the antioxidant content of the diet to prevent abnormal flavors of beef steaks and reduction in color stability.

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