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Feeding microalgae meal (All-G Rich™; *Schizochytrium limacinum* CCAP 4067/2) to beef heifers. II: Effects on ground beef color and palatability[1,2]

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ABSTRACT: The objective of this study was to examine the effects of feeding microalgae meal (All-G Rich, *Schizochytrium limacinum* CCAP 4087/2; Alltech Inc., Nicholasville, KY) to finishing heifers on 85% lean and 15% fat (85/15) ground beef PUFA content, 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 ± 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, a subset of heifers (3/pen) was harvested and the rectus femoris, vastus lateralis, vastus medialis, and vastus intermedius were collected for processing into ground beef. At 42 d postmortem, 85/15 ground beef was formulated and formed into 112-g patties and fatty acid composition, subjective palatability, and 96-h retail color stability analyses were conducted. Increasing dietary microalgae meal concentration increased ground beef 20:5n-3 and 22:6n-3 fatty acids (quadratic, $P < 0.01$). There was a treatment × hour interaction for all color attributes ($P < 0.01$). On d 0, microalgae tended ($P = 0.08$) to decrease L*, but patties had similar L* values the remainder of display ($P > 0.12$). Feeding microalgae meal affected ($P = 0.02$) b* at 24 h and decreased (linear, $P = 0.08$) b* at 48 h. From h 0 to 36 of display, microalgae affected redness of patties ($P < 0.02$), and from 48 to 72 h, microalgae meal decreased a* value (linear, $P < 0.04$). Microalgae meal did not impact sensory panel firmness, overall tenderness, or juiciness scores ($P > 0.20$) but tended to affect ($P = 0.10$) cohesiveness scores. As the amount of microalgae meal fed to heifers increased, beef flavor intensity decreased (linear, $P < 0.01$) and off-flavor intensity increased (quadratic, $P < 0.05$). Surface oxymyoglobin and metmyoglobin were impacted by microalgae meal from 12 to 36 h of display ($P < 0.01$). From 48 to 84 h of display, feeding microalgae meal to heifers decreased (linear, $P < 0.09$) surface oxymyoglobin and increased (linear, $P < 0.02$) surface metmyoglobin of patties. Although feeding microalgae meal to heifers increases the PUFA content of 85/15 ground beef, there are undesirable effects on flavor and color stability.

Key words: color, fatty acid, ground beef, microalgae, palatability

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INTRODUCTION

Givens et al. (2006) reviewed the role of meat in a healthy human diet and stated that as consumers become more affluent, their consumption of animal-derived foods increases. Americans consume 24 kg of beef per capita (USDA-ERS, 2015), 60% in the form of ground beef (Close, 2014). Beef is a major protein source that contains relatively high concentrations of SFA and is practically devoid of omega-3 fatty acids due to biohydrogenation of dietary PUFA within the rumen (Harfoot, 1978). Adequate consumption of 2 long-chain omega-3 fatty acids, eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**), which typically are found in fatty fish such as salmon and trout, reduces risk for cardiovascular disease, type 2 diabetes, and cancer (Ruxton et al., 2004; Calder, 2014).

Americans consume 2.5 kg of fish per capita annually (USDA, 2015) and because fish are the predominant source of EPA and DHA in human diets, underconsumption of fish leads to inadequate intakes of

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these important fatty acids (USDA and U.S. Department of Health and Human Services, 2010). Research has focused on manipulating the fatty acid profile of beef products as an alternative source of omega-3 fatty acids. Increasing the omega-3 fatty acid content of beef can be achieved through supplementation of forages, oilseeds, and fish-derived products (Woods and Fearon, 2009). In the first part of this study, Phelps et al. (2016) reported supplementation of microalgae meal up to 150 g/d increased EPA and DHA content of longissimus lumborum (**LL**) steaks; however, adverse fresh quality attributes such as decreased shelf life and increased off-flavors were reported. Recognizing the importance of ground beef for U.S. consumers and importance of color (Mancini and Hunt, 2005) and flavor (O'Quinn et al., 2016) on consumer acceptance of ground beef, the objective of this study was to evaluate the effect of feeding a microalgae meal on ground beef color stability and palatability characteristics.

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 for sensory panel evaluations.

Heifer Management

A more detailed description of heifer management is described in Phelps et al. (2016). Briefly, crossbred feedlot heifers (452 ± 23 kg initial BW) were assigned to pens (36 pens and 8 heifers/pen), blocked by initial pen BW (3,612 ± 177 kg), and assigned within strata to 1 of 4 treatments. Treatments consisted of 0, 50, 100, and 150 $g \cdot heifer^{-1} \cdot d^{-1}$ of supplemental microalgae meal (All-G Rich, *Schizochytrium limacinum* CCAP 4087/2; Alltech Inc., Nicholasville, KY). All treatment groups were fed a similar basal diet daily, 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. Ractopamine hydrochloride (Optaflexx; Elanco Animal Health, Greenfield, IN) was supplemented to all heifers for the final 28 d of the experiment at the rate of 400 $mg \cdot heifer \cdot^{-1} \cdot d^{-1}$.

Knuckle Collection and Ground Beef Manufacturing

Following feeding, a subset of heifers from each pen (n = 3) were harvested and fabricated at a commercial abattoir (Creekstone Farms, Arkansas City, KS). After 48 h of refrigeration, the knuckle (rectus femoris, vastus lateralis, vastus medialis, and vastus intermedius; Institutional Meat Purchase Specifications number 167; NAMP, 2010) was removed from the left side of each carcass, vacuum packaged, transported to the Kansas State University Meats Laboratory (Manhattan, KS), and stored at 2 ± 1°C until d 44 postmortem. Also, the left strip loin (Institutional Meat Purchase Specifications number 180; NAMP, 2010) was collected for the companion experiment (Phelps et al., 2016). Subcutaneous fat trimmed from the strip loins was properly identified with each individual number, vacuum packaged, and stored at 2 ± 1°C until d 44 postmortem. After storage, the knuckle of each carcass was ground separately through a 9.5-mm plate using a grinder–mixer (model 4732; Hobart Corp., Troy, OH). A 5-g sample was removed for initial fat analysis using a Univex Ground Beef Fat Analyzer (Univex Corp., Salem, NH). Using the analyzed fat percentage of the knuckle and subcutaneous fat trimmed from the same carcass's left strip loin, an 85% lean and 15% fat ground beef blend was formulated. The lean and fat portions were thoroughly hand mixed, ground through a 3.2-mm plate, and formed into 1.2-cm-thick by 10.7-cm-diameter patties weighing 112 g using an automatic patty former (Super Model 54 Food Portioner; Hollymatic Corp., Countryside, IL). Following the processing of each sample batch, the grinder and patty machine were thoroughly cleaned to prevent cross-contamination between samples. Patties used for the simulated retail display study were packaged as described below and patties (3/carcass) used for sensory analyses were blast frozen at −40°C for 30 min before being vacuum packaged and frozen at −40°C. A 500-g portion of the remaining ground beef was collected and stored at −80°C for fatty acid analyses.

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:benezene:acetyl chloride (20:27:3, vol/vol) and 2 mL of internal standard (methyl trideconoate; 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 than vortexed and centrifuged for 5 min at 500 × g at 25°C to allow separation, and the FAME in the solvent was 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 μm [i.d.];), 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°C/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; use of an internal standard is recommended in the Sukhija and Palmquist method (Sukhija and Palmquist, 1988) as the preferred method to quantify total fatty acid content.

Simulated Retail Display

One patty per blend was placed on a white 1S polystyrene foam tray with a Dri-Loc 50 absorbent pad (Cryovac Sealed Air Corp., Duncan, SC) and overwrapped with polyvinyl chloride film (AEP Industries Inc., South Hackensack, NJ) with an oxygen transmission rate of 1,450 $cm^{-3 \cdot 645}$.2 $cm^{-2 \cdot 24}$ h^{-1}. All patties were displayed in a coffin-style retail case (model DMF 8; Tyler Refrigeration Corp., Niles, MI) for 96 h under fluorescent lights (32 W Del-Warm White 3,000°K; Philips Lighting Co., Somerset, NJ) that emitted a constant 24-h case average intensity of 2,230 ± 34 lx. The case temperature was monitored using a Thermochron iButton (Maxim Integrated Products, Sunnyvale, CA), mean daily temperature of the case was 1.31 ± 3.47°C, and the case was defrosted twice daily (morning and evening) at 11°C for 30 min. Patties were rotated within the case every 12 h from left to right and front to back to account for variation in temperature and light intensity within the case. Readings for CIE L*, a*, and b* and reflectance from 400 to 700 nm were recorded every 12 h at 3 locations on each patty using a Hunter Lab Miniscan EZ spectrophotometer (Illuminant A, 2.54-cm-diameter aperture, 10° observer; Hunter Associates Laboratory, Reston, VA). 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 American Meat Science Association (**AMSA**) *Meat Color Measurement Guidelines* (AMSA, 2012). Values from the 3 scans were averaged to yield a single value for each patty.

Sensory Analyses

Sensory analyses were conducted according to procedures outlined in the AMSA meat cookery and sensory guidelines (AMSA, 2015). Twenty-four hours prior to cooking, patties were thawed on trays at 2.4 ± 1.2°C. Patties (3 per heifer) were cooked to an internal temperature of 71°C in a gas forced-air convection gas oven (model DFG-100-3; G.S. Blodgett Corp., Burlington, VA) set at 163°C. Following cooking, the 3 patties were cut into 6 wedges each and presented to a trained sensory panel with at least 6 panelists at each session. Panelists were selected from a larger pool of candidates, screened, and trained according to the AMSA meat cookery and sensory guidelines (AMSA, 2015). Selected panelists were oriented to ground beef evaluation procedures over 3 training sessions prior to the initiation of the panels. Panelists were seated at individual cubicles in a room designed for subjective sensory panel analysis and were presented 2 wedges from the 3 patties of each heifer, with 2 heifers from each treatment represented per panel. Panelists evaluated wedges for firmness, cohesiveness, overall tenderness, juiciness, beef flavor intensity, and off-flavor intensity using an 8-point scale (1 = extremely soft, not cohesive at all, extremely tough, extremely dry, extremely bland, or abundant, respectively, and 8 = extremely firm, extremely cohesive, extremely tender, extremely juicy, extremely intense, or none, respectively). A total of 12 panels were conducted to analyze all samples.

Statistical Analyses

Sensory and fatty acid data were analyzed as a randomized complete block design using PROC MIXED of SAS 9.4 (SAS Inst. Inc, Cary, NC) with pen as the experimental unit and heifer/patty as the observational unit. Treatment served as the fixed effect and block served as the random effect. Retail shelf life data were analyzed as a randomized complete block design with repeated measures. Hour of display served as the repeated measure with patty (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 hour of display for the color data. Differences were considered significant at $P \leq 0.05$ and tendencies at $0.05 > P \leq 0.10$.

RESULTS

Fatty Acid Content

Feeding increasing levels of microalgae meal to heifers increased the concentrations of 18:1 *trans*-11 and 20:2 (quadratic, $P < 0.02$) but decreased (linear, $P < 0.01$) concentrations of 18:2*n*-6 *cis* within ground beef patties (Table 1). Also, feeding increasing levels of microalgae meal tended to decrease concentrations of 18:3*n*-3 (quadratic, $P = 0.09$) and 20:3*n*-6 (quadratic, $P < 0.01$). Concentrations of 22:5*n*-3 increased (linear, $P < 0.01$) with feeding increasing levels of algae to heifers. Feeding increasing levels of microalgae in the diet

Table 1. Fatty acid profiles of 85% lean and 15% fat ground beef processed from knuckles and loin trim from heifers fed 0, 50, 100, or 150 g·heifer^{-1}·d^{-1} of microalgae meal (All-G Rich™, *Schizochytrium limacinum* CCAP 4087/2; Alltech Inc., Nicholasville, KY)

	Algae, g·heifer^{-1}·d^{-1}					*P*-value[2]		
Fatty acid methyl ester[1]	0	50	100	150	SEM	Algae	Linear	Quadratic
14:0	5.42	5.26	5.39	5.62	0.26	0.71	0.80	0.88
14:1	1.89	2.01	1.91	1.95	0.13	0.89	0.99	0.49
15:0	0.77	0.72	0.73	0.77	0.03	0.38	0.64	0.90
15:1	0.06	0.06	0.06	0.06	0.01	0.21	0.27	0.09
16:0	45.03	44.31	45.57	47.66	1.72	0.44	0.49	0.67
16:1	9.07	9.07	8.57	9.08	0.38	0.70	0.34	0.48
17:0	1.93	1.80	1.89	2.00	0.08	0.25	0.85	0.88
17:1	1.67	1.57	1.54	1.67	0.08	0.40	0.34	0.75
18:0	21.82	20.33	21.37	20.90	0.79	0.51	0.81	0.19
18:1 cis-9	67.37	65.86	65.26	66.98	2.76	0.93	0.66	0.97
18:1 trans-11	5.21	6.24	7.42	9.05	0.29	<0.01	<0.01	<0.01
18:2n-6 cis	5.55	5.24	4.69	5.24	0.18	0.01	0.01	0.44
18:2n-6 trans	0.39	0.38	0.40	0.38	0.02	0.89	0.62	0.53
18:3n-6 cis	0.03	0.03	0.03	0.04	0.01	0.08	0.59	0.96
18:3n-3	0.66	0.64	0.69	0.60	0.02	0.04	0.18	0.09
20:0	0.16	0.15	0.16	0.16	0.01	0.55	0.85	0.17
20:1	0.30	0.31	0.32	0.30	0.02	0.87	0.59	0.63
20:2	0.28	0.29	0.29	0.33	0.01	0.01	0.17	0.02
20:3n-6	0.22	0.18	0.18	0.19	0.01	<0.01	<0.01	0.01
20:4n-6 and 22:1n-9[3]	0.48	0.46	0.45	0.46	0.01	0.11	0.05	0.21
20:5n-3	0.06	0.09	0.18	0.24	6.00	<0.01	<0.01	<0.01
22:5n-3	0.16	0.15	0.19	0.21	0.01	<0.01	<0.01	0.14
22:6n-3	0.06	0.20	0.29	0.31	0.01	<0.01	<0.01	<0.01
24:0	0.09	0.10	0.10	0.10	0.01	0.12	0.08	0.30
CLA cis-9, trans-11	0.67	0.77	0.79	0.89	0.04	0.01	0.01	0.01
CLA trans-10, cis-12	0.07	0.08	0.08	0.08	0.01	0.51	0.45	0.38
CLA cis-9, cis-11	0.03	0.03	0.03	0.03	0.01	0.90	0.62	0.99
CLA trans-9, trans-11	0.21	0.24	0.25	0.29	0.01	<0.01	0.01	0.01
Total SFA[4]	75.24	72.66	75.2	77.19	2.64	0.60	0.69	0.90
Total MUFA[5]	88.19	87.70	87.58	91.69	3.38	0.74	0.88	0.56
Total PUFA[6]	8.10	8.04	7.70	8.61	0.26	0.06	0.57	0.07
Total omega-6 PUFA[7]	6.19	5.83	5.30	5.86	0.19	0.01	0.01	0.58
Total omega-3 PUFA[8]	0.93	1.086	1.25	1.45	0.03	<0.01	<0.01	<0.01
PUFA:SFA ratio[9]	0.11	0.11	0.10	0.11	0.01	0.19	0.45	0.04
Omega-6:omega-3 ratio[10]	6.75	5.38	4.24	4.02	0.13	<0.01	<0.01	<0.01
Total fatty acids	172.54	169.41	171.52	178.56	5.94	0.65	0.81	0.72

[1]Milligrams per gram wet tissue.

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

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

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

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

[6]Total 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 + CLA *trans*-9, *trans*-11.

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

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

[9]PUFA: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 + CLA *trans*-9, *trans*-11)/(14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 23:0 +24:0).

[10]0*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. Subjective cooked meat attributes of 85% lean and 15% fat ground beef patties from heifers fed 0, 50, 100, or 150 g·heifer^{-1}·d^{-1} of microalgae meal (All-G Rich™, *Schizochytrium limacinum* CCAP 4087/2; Alltech Inc., Nicholasville, KY)

	Algae, g·heifer^{-1}·d^{-1}					*P*-value[2]		
Item[1]	0	50	100	150	SEM	Trt[3]	Linear	Quadratic
Firmness	5.06	5.00	5.10	4.87	0.10	0.28	0.96	0.12
Cohesiveness	5.03	4.82	4.79	4.84	0.09	0.10	0.04	0.29
Overall tenderness	5.23	5.25	5.14	5.26	0.08	0.72	0.45	0.39
Juiciness	4.65	4.61	4.57	4.84	0.11	0.20	0.98	0.27
Beef flavor intensity	5.17	5.02	4.83	4.73	0.07	<0.01	<0.01	0.09
Off-flavor intensity	7.39	7.14	6.65	6.34	0.14	<0.01	<0.01	0.05

[1]Firmness: 1 = extremely soft and 8 = extremely firm; cohesiveness: 1 = not cohesive at all and 8 = extremely cohesive; overall 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; off-flavor intensity: 1 = abundant and 8 = none.

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

[3]Treatment effect.

increased (quadratic, $P < 0.01$) concentrations of 22:6*n*-3 and CLA *trans*-9, *trans*-11. Feeding increasing levels of microalgae meal tended (quadratic, $P < 0.07$) to increase total PUFA within ground beef patties, with a slight decrease in PUFA from 50 to 100 g/d before increasing at the 150 g/d level. Total omega-6 PUFA decreased (linear, $P < 0.01$) and total omega-3 PUFA increased (quadratic, $P < 0.01$) as the level of microalgae meal increased in the diet. The PUFA:SFA ratio was not impacted ($P > 0.19$) by feeding microalgae, but the omega-6:omega-3 ratio was reduced (quadratic, $P < 0.01$) as the level of microalgae meal in the diet increased.

Sensory Analyses

Feeding microalgae meal to heifers had no discernable effects on firmness, overall tenderness, and juiciness (Table 2; $P > 0.20$) of ground beef patties. Increased microalgae meal feeding rates tended to affect ($P = 0.10$) cohesiveness of the patties in a linear ($P = 0.04$) fashion. Beef-flavor intensity tended to decrease (linear, $P = 0.09$) and off-flavor intensity increased (quadratic, $P < 0.05$) as the inclusion level of microalgae meal in diets increased.

Simulated Retail Display

As expected, hour of display affected all color measurements ($P < 0.01$). Additionally, there were treatment × hour interactions for all color attributes ($P < 0.01$); therefore, within each hour of display, linear and quadratic contrasts were performed to focus on the impact of feeding microalgae meal. At 0 h of display, microalgae treatment tended to affect ($P = 0.08$) the L* value of ground beef patties, but during the rest of the 96-h display, there were no contrast effects on the L* value (Fig. 1; $P > 0.12$). From the onset of display through 36 h, inclusion of microalgae meal affected a* (redness) of patties ($P < 0.02$), but no linear or quadratic contrasts were detected ($P > 0.11$). From 48 to 72 h of display, increasing dietary microalgae concentration decreased the a* value (linear, $P < 0.04$). At 84 h of display, inclusion of microalgae in diets tended (linear, $P = 0.09$) to decrease the a* value, but no trends were detected. At 96 h of display, there was no effect ($P = 0.27$) of microalgae on patty redness. During the first 12 h of display, microalgae inclusion did not affect b* values ($P > 0.18$). At 24 h, feeding microalgae affected ($P = 0.02$) the b* value, but linear or quadratic contrasts were not detected ($P > 0.24$). At 36 h of display, there was no effect ($P = 0.58$) of microalgae inclusion on the b* value, but at 48 h, increasing microalgae in diets tended to decrease (linear, $P = 0.08$) b*. From h 60 through 84, microalgae inclusion did not affect b* values ($P > 0.36$). Finally, at the end of display, inclusion of microalgae tended to affect the b* value (quadratic, $P = 0.10$), with values decreasing when microalgae was included at 50 and 100 g·heifer^{-1}·d^{-1} but increasing when microalgae was included at 150 g·heifer^{-1}·d^{-1}.

Changes in patty surface oxymyoglobin and metmyoglobin percentages were partially similar to what was observed for a* values (Fig. 2). At h 0, there were no treatment effects on surface oxymyoblin and metmyoglobin percentages ($P > 0.24$). From 12 to 36 h of display, microalgae inclusion impacted surface oxymyoglobin and metmyoglobin percentages ($P < 0.02$), but no linear or quadratic contrasts were detected ($P > 0.11$). At 48 through 84 h of display, increasing microalgae meal in diets tended to decrease (h 48, 72, and 84; linear, $P < 0.09$) or decreased (h 60; $P < 0.01$) the surface oxymyoglobin percentage and increased (linear, $P < 0.02$) the surface metmyoglobin percentage on ground beef patties. Finally, at 96 h of display, treatment influenced ($P < 0.01$) the surface oxymyoglobin percentage but did not affect ($P = 0.13$) the surface metmyoglobin percentage.

DISCUSSION

Beef is a major protein source in the United States with a majority consumed in the form of ground beef (USDA, 2015). Because beef has elevated concentrations of SFA, researchers have explored nutritional regimens that increase PUFA content of beef (USDA and U.S. Department of Health and Human Services, 2010). Although supplementing heifers with microalgae meal did not increase the overall level of PUFA in ground beef, it did reduce the omega-6:omega-3 fatty acid ratio from 6.75:1 for the 0 g/d level to 4.02:1 for the 150 g/d level. This reduction to a ratio of approximately 4:1 for 150 g/d treatment brings the omega-6:omega-3 ratio to what is recommended to reduce disease conditions (Simopoulos, 2002). Previously, Givens et al. (2000) reported that feeding fish oil or fish meal effectively increased DHA and EPA in beef but also noted difficulty in maintaining sustainable and consistent quality of these products. The authors identified microalgae as an alternative to fish oil/meal. In the first portion of this study, feeding microalgae meal increased DHA and EPA content by a maximum of 850 and 340% in LL steaks, respectively (Phelps et al., 2016). In the current portion of the study, including microalgae at 150 g/d produced a similar maximum EPA content elevation (300%) but did not produce the same increase in DHA (416%). Although it is impossible to distinguish the true origins of the fatty acids in the ground beef because loin s.c. fat was used to manufacture the blends, differences in DHA response between LL steaks and 85% lean and 15% fat ground beef patties may be due to location differences from which the lean sources were harvested (loin vs. knuckle), differences in fatty acid profile of subcutaneous s.c. fat and intramuscular fat, or the percentage fat differences between loin steaks and ground beef. Little research has demonstrated differences in manipulation of the fatty acid profile of various subprimal locations of beef carcasses. Archibeque et al. (2005) demonstrated that the fatty acid profile of s.c. fat and intramuscular fat of the LM were different when corn-, flaxseed-, or sorghum-based diets were fed. Additionally, Turk and Smith (2009) demonstrated that fatty acid profiles of s.c. fat differed across locations of the beef carcass. Blackmon et al. (2015) and Kerth et al. (2015) both reported differences in the fatty acid profile of beef subprimals; however, these differences were not maintained after ground beef manufacturing. Although the magnitude of DHA increase is not similar to the LL value (Phelps et al., 2016), the increase in the ground beef product would provide more of that fatty acid when compared with a product from a nonsupplemented animal.

Increases in DHA and EPA in this study are much greater than those reported for fish oil studies. Vatansever et al. (2000) observed 96 and 75% increases for EPA and DHA, respectively, in minced forequarter muscles of steers fed fish oil. Similarly, Scollan et al. (2001) found a 110% increase in EPA content and a 90% increase in DHA content of longissimus thoracis. Mandell et al. (1997) reported a 550% increase in DHA and a 33% increase in EPA when fish meal was included at 10% of the finishing diet. Additionally, Dunne et al. (2011) reported that increasing ruminally protected fish oil in diets produced a quadratic increase in the DHA content of the neutral lipid fraction of minced neck muscles similar to the current study but did not see a response for EPA. Therefore, augmenting beef cattle finishing diets with microalgae meal is an efficient means by which to increase the DHA and EPA content of the resulting meat.

Although improving the altered fatty acid profile of ground beef patties by including microalgae in cattle finishing diets is encouraging, there are indicators of negative effects on palatability characteristics (Phelps et al., 2016). Commonly, the palatability characteristic most affected by changing the fatty acid profile is flavor. Previously, when evaluating steaks from the LL of heifers fed the microalgae product, sensory panelists indicated that texture characteristics such as tenderness and amount of connective tissue were unaffected; however, as the amount of microalgae meal increased in the diet increased, off-flavor intensity increased but beef flavor was unaffected (Phelps et al., 2016). In the current study, firmness, tenderness, and juiciness were unaffected by feeding microalgae meal to finishing heifers. Panelists tended to detect differences in cohesiveness of patties, but the differences between the treatment extremes were not large enough to be considered practically significant. Slight decreases in cohesiveness as microalgae increased in the diet may be due to the reduced melting points unsaturated fatty acids have compared with SFA. Although all patties had the same fat percentage prior to cooking, it is possible that during the cooking process the unsaturated fatty acids melted out of the patties, leading to a decreased cohesiveness. Few studies have examined manipulating the fatty acid profile of ground beef patties and the impact it has on ground beef texture. Kerth et al. (2015) reported that consumer panelists had a similar liking for the texture of ground beef patties made from the brisket, chuck, plate, flank, or round, which varied in fatty acid composition.

In agreement with panelists' steak data, off-flavor intensity increased as the level of microalgae meal included in the finishing diet increased, and this may have contributed to the observed decrease in beef-flavor intensity. When the fatty acid profile of beef products is manipulated, it is common to observe changes in flavor because omega-3 fatty acids are more susceptible to oxidation (Jacobsen, 2008). When ground beef patties were enriched with varying amounts of olive oil

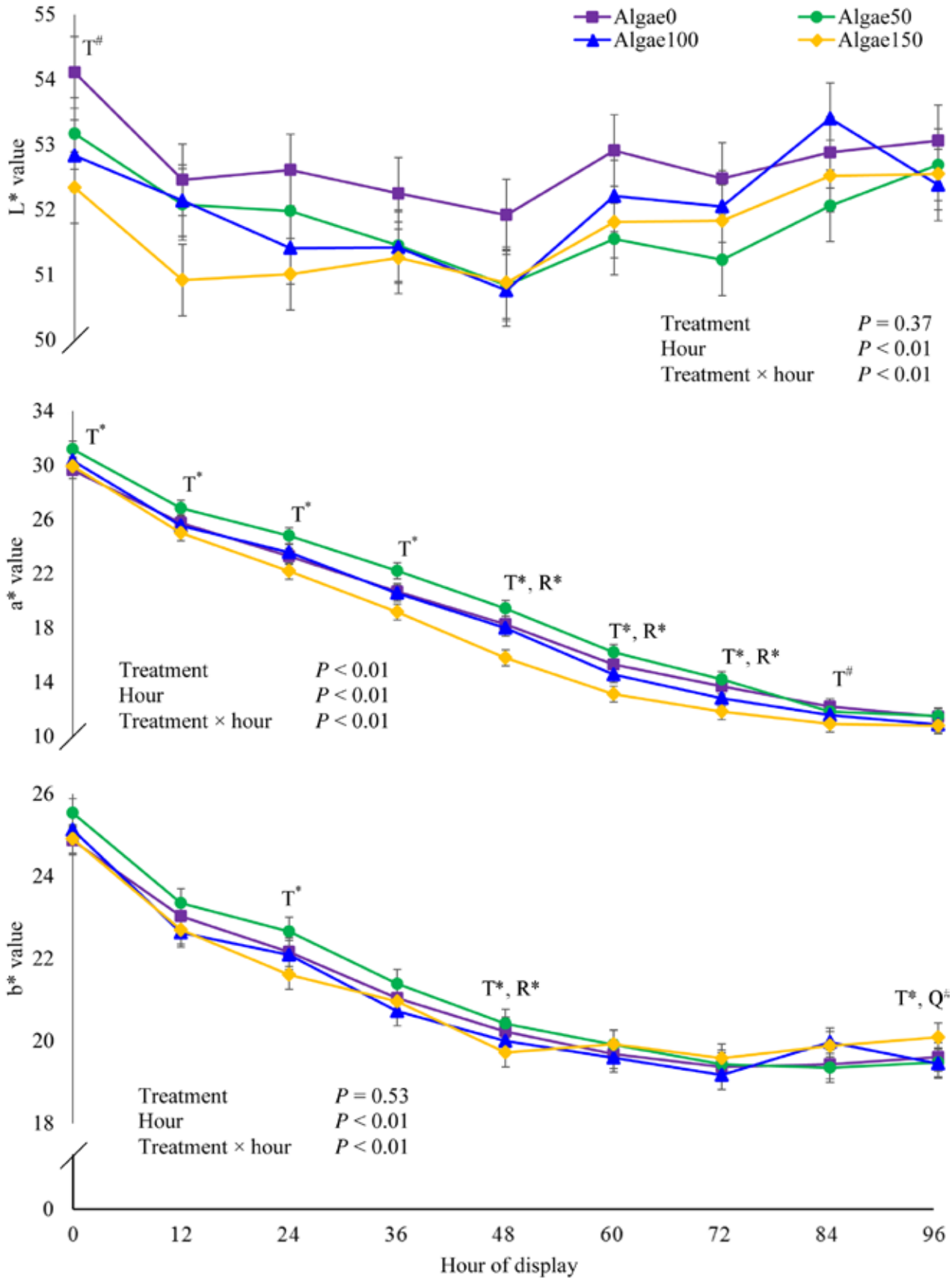

Figure 1. Ground beef patty L* (lightness; 0 = black and 100 = white), a*(redness; −60 = green and 60 = red), and b* (blueness; −60 = blue and 60 = yellow) values made from the rectus femoris, vastus lateralis, vastus medialis, and vastus intermedius of heifers supplemented 0, 50, 100, and 150 g·heifer^{-1}·d^{-1} of microalgae meal (Algae0, Algae50, Algae100, and Algae150, respectively; All-G Rich, *Schizochytrium limacinum* CCAP 4087/2, Alltech Inc., Nicholasville, KY). Patties were displayed under simulated retail conditions for 96 h. T = treatment effect; L = linear effect; Q = quadratic effect; *significant effect ($P \leq 0.05$); #tendency ($P \leq 0.10$).

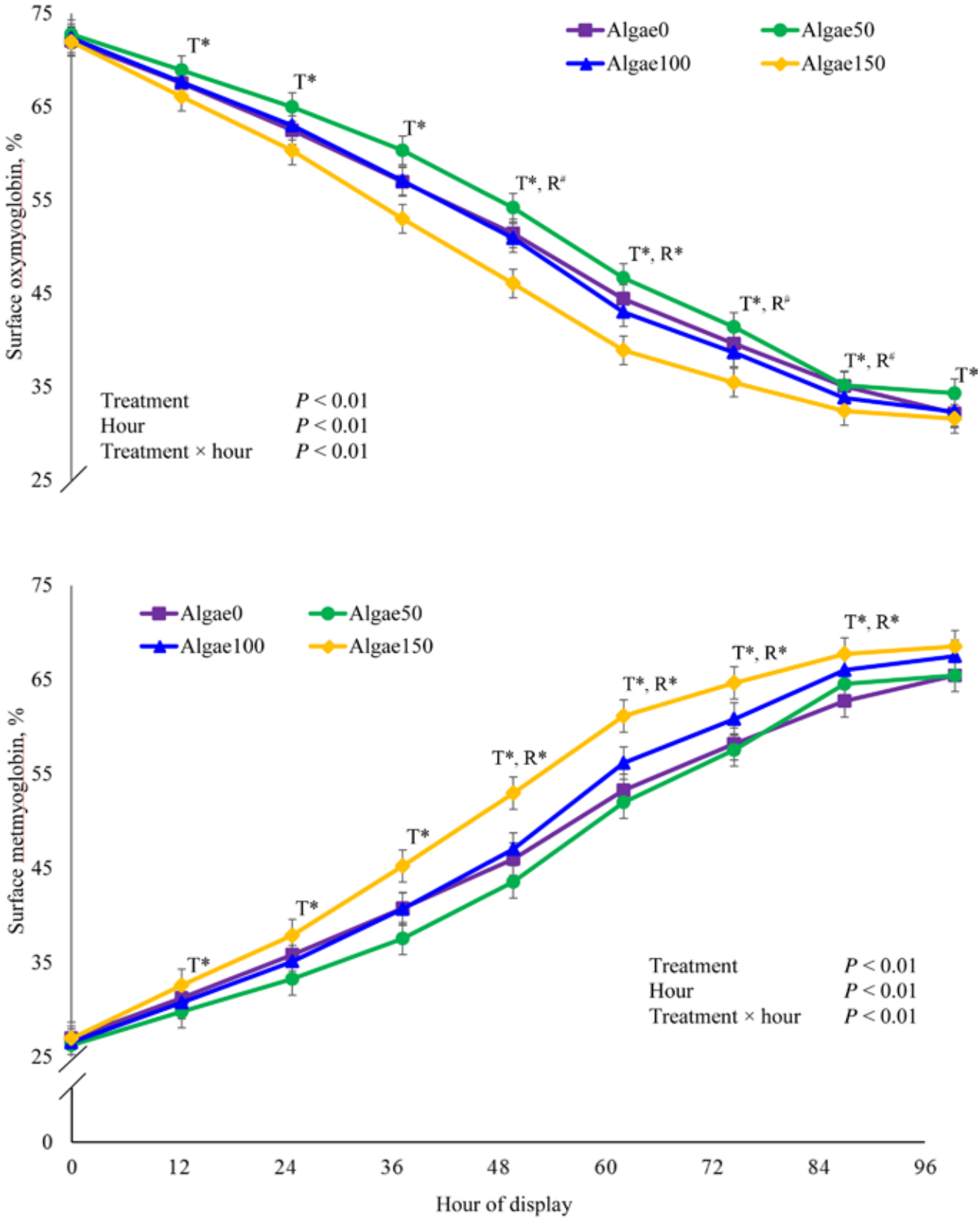

Figure 2. Ground beef patty surface oxymyoglobin and metmyoglobin percentage from the rectus femoris, vastus lateralis, vastus medialis, and vastus intermedius of heifers supplemented 0, 50, 100, and 150 g·heifer^{-1}·d^{-}1 of microalgae meal (Algae0, Algae50, Algae100, and Algae150, respectively; All-G Rich, *Schizochytrium limacinum* CCAP 4087/2, Alltech Inc., Nicholasville, KY). Patties were displayed under simulated retail conditions for 96 h and percent oxymyoglobin and metmyoglobin were calculated using the equations of Krzywicki (1979). T = treatment effect; L = linear effect; *significant effect ($P \leq 0.05$); #tendency ($P \leq 0.10$).

and EPA or DHA, Jiang et al. (2011) observed a linear decrease in beef flavor. In whole muscle products derived from cattle supplemented fish oil, increases in fishy and rancid flavors have been reported (Vatansever et al., 2000; Wistuba et al., 2006). Nute et al. (2007) reported elevated scores for "rancid" off-flavors in lamb LL when microalgae meal was included in finishing diets. The authors also reported that the decrease in lamb flavors and increases in abnormal lamb, rancid, and fishy flavors were correlated to elevated DHA and EPA content of the meat. Results of the current study and the above-cited studies indicate that dietary regimens aimed at increasing omega-3 fatty acid content can increase the incidence of off-flavors.

According to Mancini and Hunt (2005), color is the most important attribute consumers use to make purchasing decisions. When LL steaks were displayed for 7 d, feeding microalgae meal to finishing heifers accelerated formation of surface metmyoglobin during display and the effects were detected very early in the display period (Phelps et al., 2016). During the 96-h simulated display period in the current study, patties exhibited display discoloration patterns typical for ground beef (Stelzleni et al., 2013; Garner et al., 2014). Although feeding microalgae meal also affected ground beef color early in the display period, it took almost half of the display period for a linear microalgae effect on discoloration to be detected. Unlike the LL steak data, at the final period of objective color measurement, redness and surface metmyoglobin were not impacted by microalgae meal supplementation, most likely due to all patties reaching maximum metmyoglobin formation. Vatansever et al. (2000) reported that fish oil decreased the color stability of minced beef as measured by color saturation. Also, these authors and Daly et al. (2007) reported increased metmyoglobin formation on the surface of minced beef when fish oil was included in diets. In contrast, Dunne et al. (2011) reported no differences in L*, a*, and oxymyoglobin and metmyoglobin of minced neck muscle of heifers fed increasing levels of ruminally protect fish oil. Although Dunne et al. (2011) observed DHA increases within muscles that were similar to those in the current study, lack of color attribute differences may be due to being unable to increase the EPA content as in the current study. Also, Dunne et al. (2011) may not have observed differences because of the different muscle examined and because s.c. loin fat was used to achieve the proper fat amount in ground beef in the current study, whereas they added no additional fat to the minced neck muscles.

Increases in sensory panel off-flavor and discoloration of patties are likely due to increased oxidation of the long-chain PUFA. Beef products with a more unsaturated fatty acid profile are more susceptible to oxidation during postmortem storage and retail display (Yang et al., 2002). Using thiobarbituric acid reactive substances (**TBARS**) as an oxidation measurement, Phelps et al. (2016) reported a quadratic increase in TBARS value of LL steaks at the beginning and end of display as microalgae meal inclusion increased. Dunne et al. (2011) reported increases in TBARS with increased concentration in the diet of heifers. Although TBARS were not measured in this study, it is reasonable to hypothesize that as microalgae meal inclusion increased in finishing diets, oxidation of beef patties was increased, causing increased discoloration and sensory panel off-flavors. Literature indicates that feeding elevated levels of antioxidants, such as vitamin E, during the finishing phase can alleviate lipid oxidation and color stability issues (Yang et al., 2002; Gobert et al., 2010). Future research using this microalgae product or other similar products should focus on including antioxidants in finishing diets to prevent the negative color stability and flavor profiles of the meat products derived from cattle fed.

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