Performance, insulin sensitivity, carcass characteristics, and fatty acid profile of beef from steers fed microalgae¹

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ABSTRACT: Heterotrophic production of microalgae biomass provides a consistent, high-quality source of docosahexaenoic acid (DHA; C22:6 n-3) in triglyceride oils that could be used as a ration supplement for feedlot steers to improve nutritional qualities of beef. Sixty Angus × Simmental steers (438 ± 6.4 kg) were allotted to two treatments (30 steers each, six pens, five steers/pen) to determine the effects of ForPLUS (DHA-rich microalgae *Aurantiochytrium limacinum*; 63.6% fat; 17.9% DHA; 30 mg/kg Sel-Plex; Alltech Inc.) on performance, insulin sensitivity, LM fatty acid composition, and meat quality. Steers were fed basal diets containing 45% corn, 30% distillers dried grains with solubles, 20% corn silage, and 5% supplement. Basal diets were formulated to contain 16.1% CP and 1.32 Mcal/kg NE_g. Treatments were delivered to steers in a ground corn-based top-dress (454 g total/steer) and contained no microalgae for control steers or 100 g/steer daily of ForPLUS for microalgae steers. A glucose tolerance test (GTT) was performed 10 d prior to slaughter. Steers were slaughtered when a target pen BW of 621 kg was achieved. Fatty acid oxidation potential was determined by measuring thiobarbituric acid reactive substances (TBARS)

on LM samples collected 24 h after slaughter and aged for 48 h or 21 d. Weight and BW gain did not differ during the study ($P \geq 0.13$); however, steers fed microalgae remained in the feedlot seven more days compared to steers fed the control diet (111 vs. 104 d; $P = 0.04$). Overall DMI decreased ($P = 0.002$) and G:F increased during the second half of the study ($P = 0.04$) in steers fed microalgae compared to steers fed the control diet. Steers fed microalgae secreted less insulin ($P = 0.01$) and took longer to clear glucose ($P = 0.01$) during a 2-h GTT. Carcass traits did not differ between treatments ($P \geq 0.23$). Microalgae had no effect on n-6 content ($P = 0.67$), but more than doubled the n-3 fatty acid percentage and the n-3:n-6 ratio of the LM ($P < 0.0001$). The percentage of n-3 fatty acids C20:5 and C22:6 were increased ($P < 0.0001$) 4-fold and 6.25-fold, respectively, by microalgae supplementation. Concentration of TBARS did not differ in LM aged for 48 h ($P = 0.91$); however, when aged for 21 d, steers fed microalgae tended to produce LM with greater TBARS concentration compared to steers fed the control diet ($P = 0.08$). In conclusion, DHA-rich microalgae decreased DMI of steers, and increased n-3 fatty acids and beef oxidation in steaks aged for 21 d.

Key words: beef, carcass characteristics, growth, insulin sensitivity, microalgae, omega-3 fatty acids

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INTRODUCTION

Long-chain omega-3 (n-3) PUFA has been reported to improve insulin sensitivity in cattle (Cartiff et al., 2013). Because depressed insulin sensitivity is associated with decreased amino acid use for protein synthesis (Wray-Cahen et al., 1997) and increased extra-muscular fat deposition (Trenkle and Topel, 1978; Kahn and Flier, 2000), nutritional strategies that improve insulin sensitivity are of interest for beef production. In addition, a diet rich in PUFA, particularly n-3, has beneficial effects on human health, as these fatty acids can aid in the prevention of atherosclerosis, insulin resistance, neurological dysfunction, and autoimmune disorders (Calder, 2001). However, ruminant fat is naturally almost devoid of n-3 PUFA, specifically eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3).

Fish oil is the most common ration additive used to provide n-3 fatty acids to ruminants. However, fish do not synthesize n-3 fatty acids; they consume microscopic algae or other algae-consuming fish to obtain n-3 fatty acids. New technology allows for heterotrophic production of microalgae in bulk fermenters that do not require the lighting and electricity previously needed for phototrophic microalgae growth (Harel et al., 2002). Microalgae biomass produced in these facilities provides a consistent high-quality source of DHA that could be used as a ration supplement for beef cattle, particularly because n-3 PUFA in microalgae are less biohydrogenated in the rumen compared to fish oil (Cooper et al., 2004). We hypothesized that inclusion of microalgae would improve insulin sensitivity, performance, and carcass characteristics of steers and would increase n-3 PUFA content of beef. Therefore, the objectives of this study were to quantify the effects of microalgae on performance, insulin sensitivity, and carcass characteristics of feedlot steers and characterize changes in fatty acid composition and oxidative stability of meat.

MATERIALS AND METHODS

Research protocols using animals followed guidelines in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010) and were approved by the Purdue Animal Care and Use Committee.

Animals, Experimental Design, and Diets

Sixty Angus × Simmental steers (initial BW = 438 ± 6.4 kg) were used at the Beef Unit of

the Animal Science Research and Education Center (ASREC) of Purdue University from December 2013 to June 2014. Steers were weighed on two consecutive days for initial BW determination, blocked by BW and breed composition, and allotted by block to 12 pens (six pens/treatment and five steers/pen) in a randomized complete block design. Pens (6.1 × 3.3 m) were located in a curtain-sided, slatted-floor finishing barn, and provided 122 cm of bunk space per animal.

Steers were vaccinated against bovine rhinotracheitis, bovine viral diarrhea, parainfluenza-3, and bovine respiratory syncytial virus (Bovi-Shield GOLD FP 5; Zoetis Animal Health, Florham Park, NJ), against *Haemophilus somnus*, *Pasteurella*, and *Clostridia* (Vision-7 Somnus; Merck Animal Health, Summit, NJ), and treated with an anthelmintic (Valbazen; Zoetis Animal Health) for internal and external parasites at weaning and upon arrival at the ASREC feedlot. Steers were implanted with Revalor-XS (40 mg estradiol and 200 mg trenbolone acetate; provided courtesy of Merck Animal Health) at feedlot entry, and assigned to either 1) a control treatment with no microalgae product or 2) a microalgae treatment with 100 g/animal daily of ForPLUS (DHA-rich microalgae *Aurantiochytrium limacinum*; 63.6% fat; 17.9% DHA; 30 mg/kg Sel-Plex) provided courtesy of Alltech Inc. (Nicholasville, KY). Treatments were mixed in a ground corn-based top-dress delivered to bunks at a rate of 454 g/steer daily. The control top-dress contained 3 mg/d of selenium as sodium selenite to equalize selenium concentration between treatments. The microalgae were grown heterotrophically in a unique process on a fresh water, low-sodium media. The basal diet was the same except for the composition of the top-dress and was formulated to meet or exceed NRC (1996) requirements for protein, energy, vitamins, and minerals (Table 1). Total mixed rations were fed once daily at 0800 h and steers were allowed ad libitum access to feed and water. Daily feed deliveries were adjusted using the South Dakota State University 4-point bunk scoring system (Pritchard, 1993) to allow for ad libitum feed intake with little or no accumulation of unconsumed feed. Feed delivery was recorded daily and feed samples were collected bi-weekly and oven-dried at 55 °C for 3 d for DM determination (AOAC, 1990). Dried samples were ground using a standard Wiley laboratory mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA), and composited at the end of the experiment for analysis of CP, NDF, ADF, and minerals (Ca, P, Mg, K, S).

Table 1. Diet composition¹

Ingredients (% of DM)	
Corn	45.0
Dried distillers grains with solubles	30.0
Corn silage	20.0
Vitamin/mineral supplement ²	5.0
Nutrient composition	
CP, %	16.1
NE _m , Mcal/kg	2.22
NE _g , Mcal/kg	1.33
Calcium, %	0.85
Phosphorus, %	0.54
Potassium, %	0.87
Sulfur, %	0.29

Abbreviation: DHA = docosahexaenoic acid.

¹Treatments were 1) control: ground corn top-dress (454 g/steer/d) with no microalgae or 2) microalgae: ground corn top-dress (454 g/steer/d) with 100 g/steer daily as ForPLUS (DHA-rich microalgae *Aurantiochytrium limacinum*; 63.6% fat; 17.9% DHA; 30 mg/kg Sel-Plex; provided courtesy of Alltech Inc., Nicholasville, KY). The control top-dress contained 3 mg/d Se as sodium selenite to equalize Se concentrations.

²Vitamin/mineral premix contained (DM basis): 15.36% Ca, 0.39% Mg, 0.92% K, 0.17% S, 3.55 ppm Co, 160.56 ppm Cu, 8.96 ppm I, 449.18 ppm Fe, 406 ppm Mn, 6.32 ppm Se, 501.16 ppm Zn, 38.4 IU/g vitamin A, 4.55 IU/g vitamin D, 134 IU/kg vitamin E, 386.5 ppm Rumensin (176.4 g/kg, Elanco Animal Health, Indianapolis, IN), 119.3 ppm Tylan (88.2 g/kg, Elanco Animal Health).

Growth, Feedlot Performance, and Carcass Data

Steers were weighed every 25–30 d during the experiment to monitor ADG and on two consecutive days at the onset of the experiment and prior to slaughter to determine initial and final BW, respectively. Body weights were taken prior to feeding. Average daily gain, DMI, and G:F were determined from days 0 to 54 (period 1), day 55 to slaughter (period 2), and day 0 to slaughter (overall). Animals were slaughtered at a commercial packing facility (Tyson Foods, Joslin, IL) when average pen BW of 621 ± 10.7 kg was achieved. Hot carcass weight and dressing percentage were determined after slaughter and before chilling. After carcasses were chilled for 24 h, the following measurements were obtained by qualified university personnel: subcutaneous fat thickness (cm) taken between the 12th and 13th ribs, LM area (cm²) obtained by direct grid reading between the 12th and 13th ribs, KPH as a percentage of HCW, as well as marbling score, and USDA quality and yield grades (USDA, 1997).

Intravenous Glucose Tolerance Test

An intravenous GTT (IVGTT) was performed on 24 steers (two steers per pen; six pens per treatment) 10 d prior to slaughter. The average BW of

the steers chosen was equal to the pen average BW. Steers were removed from feed for 12 h but maintained ad libitum access to water. On the morning of the IVGTT, steers were weighed to determine the amount of glucose infusion. Catheters were then inserted into the jugular vein of each steer and secured using aseptic procedures. Steers were immediately placed in stanchions and allowed approximately 2 h to recover. Blood samples were obtained at 10 and 5 min prior to glucose bolus infusion for determination of fasting blood glucose and insulin concentrations. At time 0, a bolus dose of glucose was delivered in a 50% dextrose solution (Agripharm Products, Westlake TX) at a rate of 0.25 g of glucose/kg of BW. Subsequent blood samples were then collected at 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, and 120 min after bolus infusion for analysis of glucose and insulin concentrations. Prior to each sample, 5 mL of blood was drawn into a syringe and discarded to clear the catheter. A new syringe was then used to collect an additional 5 mL of blood that was placed in a NaF-coated tube (BD Vacutainer; Becton-Dickinson, Franklin Lakes, NJ) to arrest glucose metabolism and the tube was immediately placed on ice. The catheter was flushed with 3 mL of a sterile 3.5% Na citrate solution to prevent clotting and clear the infusion line. Blood samples were centrifuged immediately after the conclusion of the IVGTT at $1,750 \times g$ for 25 min at 4 °C; plasma was recovered, transferred to 5 mL polystyrene tubes, and frozen at –20 °C until analyzed. Plasma was analyzed for insulin using an Iodine¹²⁵ radioimmunoassay kit (Coat-A-Count Insulin, Siemens Medical Solutions Diagnostics, Malvern, PA) and a gamma counter (Cobra II Auto-Gamma; Packard Instrument Co., Downers Grove, IL). Glucose concentration was determined in plasma using a glucose oxidase/peroxidase enzyme kit (Glucose Liquicolor Procedure No. 1070, Stanbio Laboratory, Boerne, TX) and absorbance was read at a wavelength of 495 nm with an Opsys MR microplate reader (Dynex Technologies Inc., Chantilly, VA). Nonesterified fatty acid concentration was determined in plasma using an Acyl-CoA synthetase/Acyl-CoA oxidase enzyme kit (NEFA C Kit, Wako Diagnostics Inc., Richmond, VA) and absorbance was read at a wavelength of 550 nm with the previously described microplate reader.

Plasma and Muscle Fatty Acid Profile

Blood samples were taken from steers at feedlot entry (day 0) and at slaughter (day 108) for analysis

of plasma fatty acid content. Blood samples were collected in BD Vacutainer tubes containing 158 USP Sodium Heparin (Becton Drive, Franklin Lakes, NJ), inverted, then placed on ice until centrifugation at $3,000 \times g$ for 20 min at 4 °C. Plasma was separated and stored at -20 °C until fatty acid analysis. Lipid was extracted from plasma according to the procedures of [Sukhija and Palmquist \(1988\)](#). Freeze-dried plasma samples (25 mg) were weighed into culture tubes and 1 mL of benzene containing a C13 internal standard (50 µg C13 in 1 mL of benzene), 1 mL of benzene, and 3 mL of freshly made 5% methanolic HCl (prepared by slowly adding 10 mL of acetyl chloride to 100 mL of anhydrous methanol) was added. The culture tubes were vortexed for 1 min at a slow speed so that the material remained 2–3 cm from the bottom. Tubes were heated for 2 h in a heat block at 90 °C, cooled to room temperature, and 5 mL of a 6% K₂CO₃ was added, followed by 1 mL of hexane. Contents were centrifuged for 10 min at $500 \times g$ and the upper organic solvent layer was transferred to another tube to which 1 g of sodium sulfate was added. Contents were again centrifuged at $500 \times g$ for 10 min and the upper hexane layer was transferred to 1.5 mL vials.

To determine fatty acid composition of muscle, LM samples from the left side of the carcass at the 12th–13th rib interface were collected 24-h post-slaughter. Lipids were extracted from the LM samples according to the procedures of [Bligh and Dyer \(1959\)](#), quantified, and derivatized as methyl esters by a sodium methoxide catalyzed methanolysis procedure according to the method of [Christie \(1982\)](#) as modified by [Chouinard et al. \(1999\)](#).

The methyl esters of fatty acids from plasma beef were quantified on an Agilent 7890A gas chromatograph equipped with an automatic injector and flame ionization detector (Agilent Inc.; Santa Clara, CA). A 100 m SLB-IL111 column (100 m $\times$ 0.25 mm i.d. $\times$ 0.20 µm film thickness; Supelco Inc., Bellefonte, PA) was used according to the procedure of [Delmonte et al. \(2011\)](#) to allow for separation of all long-chain fatty acid (LCFA) isomers. Fatty acid molar percentage was calculated by dividing individual fatty-acid concentrations by the total of all fatty-acid concentrations.

Lipid Oxidation

Lipid oxidation was determined by the modified thiobarbituric acid reactive substances (TBARS) method of [Buege and Aust \(1978\)](#). Minced sample (5 g) was placed in a 50 mL test tube and

homogenized with 15 mL deionized distilled water using an Ultraturrex homogenizer for 15 s at high speed. The meat homogenate (1 mL) was transferred to a disposable test tube (13 $\times$ 100 mm) and butylated hydroxytoluene (7.2%, 50 mL) and a thiobarbituric acid/trichloroacetic acid (TBA/TCA) solution (2 mL) was added. The mixture was vortexed and then incubated in a 90 °C water bath for 15 min to develop color. After cooling for 10 min in cold water, the sample was centrifuged at $2,000 \times g$ for 15 min at 4 °C. Absorbance of the resulting upper layer was determined at 531 nm against a blank containing 1 mL deionized distilled water and 2 mL TBA/TCA solution. Samples were read using the previously described microplate reader. The TBARS values were calculated from a standard curve and expressed as mg of malondialdehyde per kg of original meat.

Statistical Analysis

Data were analyzed as a complete randomized block design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), and pen was considered the experimental unit. For performance data, the model included the random effects of block and pen, and the fixed effects of microalgae inclusion and d, as well as the microalgae $\times$ d interaction. For the IVGTT data, the model included the random effects of block and pen, and the fixed effects of microalgae inclusion and time, as well as the microalgae $\times$ time interaction. Performance and IVGTT data were analyzed as repeated measures by comparing four covariance structures for each variable (compound symmetric, autoregressive order one, heterogeneous autoregressive order one, and unstructured) and the covariance structure that yielded the smallest Bayesian information criterion was used for the results presented. Carcass characteristics were analyzed using MIXED procedure of SAS as a complete randomized block design and the model included the random effects of block and pen, and the fixed effect of microalgae inclusion. The least squares means (LSMEANS) statement was used to calculate the adjusted means for treatments. For all variables analyzed, a *P* value ≤ 0.05 was identified as significantly different, while $0.05 < P \leq 0.10$ was identified as a tendency.

RESULTS AND DISCUSSION

Steers fed microalgae had decreased DMI ($P \leq 0.002$), no change in ADG ($P \geq 0.13$), and had improved G:F during period 2 ($P = 0.04$) ([Table 2](#)).

Table 2. Effect of microalgae on performance of steers

Item	Treatment ^{1,2}		SEM	P value
	Control	Microalgae		
BW, kg				
Day 0	438.7	438.1	6.4	0.94
Day 54	560.3	548.1	6.0	0.18
Day 108	621.3	621.6	10.7	0.98
ADG, kg/d				
First half (days 0 to 54)	2.25	2.03	0.097	0.13
Second half (days 55 to 108)	1.18	1.26	0.097	0.53
Overall (days 0 to 108)	1.75	1.66	0.097	0.50
DMI, kg/d				
First half (days 0 to 54)	11.61	10.38	0.240	0.001
Second half (days 55 to 108)	11.18	10.02	0.240	0.002
Overall (days 0 to 108)	11.42	10.21	0.240	0.002
G:F, kg/kg				
First half (days 0 to 54)	0.194	0.195	0.0069	0.91
Second half (days 55 to 108)	0.105	0.126	0.0069	0.04
Overall (days 0 to 108)	0.153	0.162	0.0069	0.38
Days on feed	104	111	2.0	0.04

¹Treatments were 1) control: ground corn top-dress (454 g/steer daily) with no microalgae or 2) microalgae: ground corn top-dress (454 g/steer daily) with 100 g/steer daily as ForPLUS (Alltech Inc., Nicholasville, KY). The control top-dress contained 3 mg/d Se as sodium selenite to equalize Se concentrations.

²Six pens/treatment; five steers/pen.

However, dietary microalgae increased the number of days it took for steers to reach slaughter BW ($P = 0.04$). Meale et al. (2014) observed no impact of up to 3% of the diet DM as the *Schizochytrium* microalgae species on ADG in lambs and Stokes et al. (2016) reported that a deoiled algae meal + soybean hull product (43:57) also did not impact ADG in feedlot steers fed a 10% hay diet. In addition, Shingfield et al. (2010) observed that up to 2.4% of the diet DM as fish oil did not impact ADG in steers fed corn silage diets. However, in contrast to the current study, Meale et al. (2014) observed no effect of algae on DMI in lambs and Stokes et al. (2016) reported a linear increase in DMI in feedlot steers. In dairy cows, Franklin et al. (1999) observed a 20% decrease in DMI without an effect on milk yield using the same microalgae species (*Aurantiochytrium*). Boeckaert et al. (2008) observed a reduction in DMI in dairy cows supplemented with 1% of the diet DM of the *Schizochytrium* microalgae and also noted a decrease in milk production. Fish oil has decreased DMI in beef cattle in some studies (Lee et al., 2008; Shingfield et al., 2010), but not others (Lee et al., 2005; Kim et al., 2008). It is possible in the current study that the decrease in DMI was because microalgae impaired ruminal microbial fiber digestion (Zinn and Plascencia, 1996). However, it may be more likely that the PUFA contained in the microalgae impaired DMI by affecting other factors

such as dietary palatability, gut motility, and release of gut hormones that control satiety (Allen, 2000). Nevertheless, the decrease in DMI did not affect ADG in the present study, particularly in period 2 and resulted in a 20 and 5.9% improvement in G:F for period 2 and overall. It may be possible that cattle fed microalgae were more efficient than cattle fed the control diet because they were in a healthier immune state. Eicosapentaenoic acid and DHA can attenuate inflammatory responses by promoting the synthesis of prostaglandin E_3 (PGE_3) rather than prostaglandin E_2 (Schmitz and Ecker, 2008). Prostaglandin E_2 is a potent stimulator of the acute-phase and other inflammatory responses, whereas PGE_3 is not (Yaqoob and Calder, 2007).

Because insulin resistance is associated with decreased amino acid use for protein synthesis (Wray-Cahen et al., 1997) and increased fat deposition (Trenkle and Topel, 1978; Kahn and Flier, 2000), nutritional strategies that improve insulin sensitivity are of interest for beef cattle production. Long-chain n-3 PUFAs have been reported to improve insulin sensitivity in cattle (Cartiff et al., 2013). In addition, when steers were infused abomasally with fish oil, which is high in DHA and EPA, insulin sensitivity improved and protein breakdown and amino acid oxidation decreased (Gingras et al., 2007). Results from the IVGTT in the present study are presented in Fig. 1–4. Dietary microalgae increased plasma

Figure 1. Effect of microalgae on plasma glucose concentration during a glucose tolerance test. The symbol (*) indicates a response ($P \leq 0.05$) of plasma glucose to microalgae inclusion after glucose infusion. Treatments were 1) control: ground corn top-dress (454 g/steer daily) with no microalgae or 2) microalgae: ground corn top-dress (454 g/steer daily) with 100 g/steer daily as ForPLUS (Alltech Inc., Nicholasville, KY). The control top-dress contained 3 mg/d Se as sodium selenite to equalize Se concentrations. SEM = 9.6 mg/dL. $n = 6$ pens/treatment, 2 steers selected/pen.

Figure 2. Effect of microalgae on plasma insulin concentration during a glucose tolerance test. The symbol (*) indicates a response ($P \leq 0.05$) of plasma insulin to microalgae inclusion after glucose infusion. Treatments were 1) control: ground corn top-dress (454 g/steer daily) with no microalgae or 2) microalgae: ground corn top-dress (454 g/steer daily) with 100 g/steer daily as ForPLUS (Alltech Inc., Nicholasville, KY). The control top-dress contained 3 mg/d Se as sodium selenite to equalize Se concentrations. SEM = 5.4 μIU/mL. $n = 6$ pens/treatment, 2 steers selected/pen.

glucose at 45 and 90 min ($P \leq 0.03$) and tended to increase plasma glucose at 75 and 120 min ($P \leq 0.09$) after glucose infusion. Dietary microalgae decreased plasma insulin at 10 and 20 min ($P \leq 0.04$) and tended to decrease plasma insulin at 15 min ($P = 0.06$) after glucose infusion. As a result, the insulin:glucose ratio decreased in plasma from steers supplemented with microalgae at 10, 15, 20, and 25 min after glucose infusion ($P \leq 0.05$).

Decreased clearance of glucose from the bloodstream, as seen in the present study, is generally an indication that tissues are less sensitive to insulin-mediated glucose uptake. However, decreased circulating insulin is an indication of improved insulin sensitivity. In agreement with the present study, fish oil was reported to increase blood glucose in dairy cows (Kupczynski et al., 2012) and decrease circulating insulin in rats (Chicco et al., 1996; D'Alessandro et al., 2002). Although plasma

Figure 3. Effect of microalgae on plasma insulin:glucose concentration during a glucose tolerance test. The symbol (*) indicates a response ($P \leq 0.05$) of plasma insulin/glucose to microalgae inclusion after glucose infusion. Treatments were 1) control: ground corn top-dress (454 g/steer daily) with no microalgae or 2) microalgae: ground corn top-dress (454 g/steer daily) with 100 g/steer daily as ForPLUS (Alltech Inc., Nicholasville, KY). The control top-dress contained 3 mg/d Se as sodium selenite to equalize Se concentrations. SEM = 4.1 pmol/L:mmol/L. $n = 6$ pens/treatment, 2 steers selected/pen.

Figure 4. Effect of microalgae on plasma NEFA concentration during a glucose tolerance test. Plasma NEFA did not differ at any time point between treatments ($P \geq 0.31$). Treatments were 1) control: ground corn top-dress (454 g/steer daily) with no microalgae or 2) microalgae: ground corn top-dress (454 g/steer daily) with 100 g/steer daily as ForPLUS (Alltech Inc., Nicholasville, KY). The control top-dress contained 3 mg/d Se as sodium selenite to equalize Se concentrations. SEM = 0.03 mg/dL. $n = 6$ pens/treatment, 2 steers selected/pen.

NEFA concentrations during the IVGTT in the present study were not affected by microalgae supplementation ($P \geq 0.31$), total plasma NEFA on day 108 (Table 3) was increased by microalgae supplementation ($P = 0.05$). Previous studies in rats have demonstrated that dietary fish oil can promote lipid oxidation in tissues (Baker et al., 2000). The fact that insulin was decreased and glucose was elevated

during the IVGTT in the present study in combination with increased circulating NEFA at day 108, indicates that dietary microalgae may have shifted energy utilization in tissues from glucose to fat, thus sparing glucose and impacting its uptake into tissues.

Plasma fatty acid composition is presented in Table 3. Concentration of C16:1 in plasma on day

Table 3. Effect of microalgae on plasma fatty acids

Tab	Treatment ^{1,2}							
	Control		Microalgae		SEM	Within day <i>P</i> value		Trt × d
	Day 0	Day 108	Day 0	Day 108		Day 0	Day 108	
Fatty acid ³								
Total NEFA ⁴	242.4	221.7	233.1	250.7	9.84	0.51	0.05	0.03
C10:0	0.03	0.03	0.04	0.03	0.006	0.25	0.92	0.49
C12:0	0.07	0.06	0.08	0.06	0.010	0.48	0.98	0.13
C14:0	1.21	1.11	1.33	1.14	0.140	0.55	0.91	0.54
C14:1	0.02	0.03	0.03	0.02	0.007	0.32	0.69	0.55
C15:0	0.78	0.63	0.78	0.64	0.029	0.99	0.83	<0.0001
C16:0	17.71	16.18	17.95	16.61	0.845	0.84	0.72	0.25
C16:1 <i>cis</i> -9	0.73	0.83	1.16	1.00	0.126	0.02	0.36	0.48
C17:0	1.36	1.17	1.29	1.06	0.055	0.38	0.15	<0.01
C17:1	0.13	0.10	0.12	0.09	0.007	0.76	0.10	<0.0001
C18:0	18.07	19.24	18.76	16.79	0.732	0.51	0.02	0.10
C18:1 <i>trans</i> -9	2.07	1.87	2.34	2.26	0.294	0.53	0.36	0.85
C18:1 <i>cis</i> -9	4.86	4.38	5.09	3.64	0.265	0.55	0.05	<0.001
C18:2	46.00	47.57	44.62	48.27	1.653	0.56	0.77	0.25
C18:3n-3	0.58	0.54	0.60	0.65	0.026	0.50	<0.01	0.27
C18:3n-6	0.34	0.19	0.34	0.09	0.029	0.95	0.01	<0.0001
C20:0	0.15	0.16	0.17	0.14	0.017	0.37	0.44	0.20
C20:1	0.48	0.51	0.05	0.05	0.330	0.36	0.33	0.99
C20:2	0.11	0.10	0.12	0.13	0.021	0.74	0.24	0.80
C20:3n-3	0.00	0.00	0.01	0.04	0.004	0.31	<0.0001	<0.0001
C20:3n-6	1.64	1.27	1.49	0.57	0.117	0.36	<0.0001	<0.0001
C20:4	2.15	2.24	2.01	2.14	0.112	0.39	0.51	0.62
C20:5	0.19	0.12	0.21	2.36	0.182	0.96	<0.0001	<0.0001
C22:1	0.10	0.08	0.04	0.03	0.044	0.34	0.40	0.93
C22:2	0.23	0.04	0.03	0.06	0.014	0.64	0.21	0.22
C22:6	0.12	0.14	0.21	1.05	0.047	0.20	<0.0001	<0.0001
C23:0	0.23	0.31	0.28	0.29	0.023	0.14	0.62	0.01
C24:0	0.08	0.17	0.09	0.17	0.033	0.89	0.95	0.03
C24:1	0.26	0.38	0.29	0.23	0.071	0.79	0.14	0.40
MCFA ⁵	2.19	1.92	2.34	1.95	0.181	0.55	0.91	0.19
LCFA ⁶	97.82	98.08	97.66	98.05	0.181	0.55	0.91	0.19
Total SFA	40.00	39.32	41.08	37.26	1.591	0.63	0.36	0.24
Total MUFA	8.30	7.91	9.21	7.36	0.494	0.20	0.44	0.01
Total PUFA	51.22	52.27	49.66	55.34	1.741	0.53	0.22	0.08
PUFA:SFA	1.33	1.37	1.23	1.54	0.101	0.49	0.24	0.10
n-3 fatty acids	0.89	0.80	1.03	4.10	0.215	0.67	<0.0001	<0.0001
n-6 fatty acids	4.25	3.84	3.94	2.90	0.227	0.35	<0.01	0.01
n-3:n-6	0.22	0.21	0.26	1.40	0.047	0.48	<0.001	<0.0001

¹Treatments were 1) control: ground corn top-dress (454 g/steer daily) with no microalgae or 2) microalgae: ground corn top-dress (454 g/steer daily) with 100 g/steer/d as ForPLUS (Alltech Inc., Nicholasville, KY). The control top-dress contained 3 mg/d Se as sodium selenite to equalize Se concentrations.

²Six pens/treatment; five steers/pen.

³Molar percentage.

⁴Nonesterified fatty acids, mmol/mL.

⁵Medium-chain fatty acids (≤15:1).

⁶Long-chain fatty acids (≥16:0).

0 was greater ($P = 0.02$) for steers fed microalgae compared to steers not fed microalgae. The reason for such a difference is unclear. All other plasma fatty acids did not differ between treatments at feedlot entry ($P \geq 0.14$). At slaughter, concentration of medium-chain fatty acids (MCFA) and

LCFA in plasma did not differ due to microalgae supplementation ($P = 0.91$). Total SFA, MUFA, PUFA, or PUFA:SFA ratio did not differ at individual time points ($P \geq 0.20$); however, there was a treatment × day interaction for MUFA ($P = 0.01$) and a tendency for a treatment × day interaction

for PUFA ($P = 0.08$) and PUFA:SFA ($P = 0.10$). Monounsaturated fatty acids decreased and PUFA and the PUFA:SFA ratio increased from days 0 to 108; however, the magnitude of change was greater in plasma from steers fed microalgae compared to plasma from steers fed the control diet. Specific fatty acids in plasma affected by microalgae included 18:0 and *cis*-9 18:1, which were decreased as a result of microalgae supplementation ($P \leq 0.05$). Duckett and Gillis (2010) and Shingfield et al. (2010) similarly reported decreased duodenal flow of 18:0 with fish oil supplementation and increased flow of biohydrogenation intermediates. However, dietary fish oil had no effect (Duckett and Gillis, 2010; Shingfield et al., 2010) or increased (Lee et al., 2005, 2008) duodenal flow of *cis*-9 18:1. In contrast, and in agreement with the present study, Kim et al. (2008) reported that fish oil decreased duodenal flow of *cis*-9 18:1. Omega-3 PUFAs found in algae and fish oil inhibit the final step of biohydrogenation, which is the conversion of 18:1 *trans*-11 to 18:0 (AbuGhazaleh and Jenkins, 2004), thus providing a basis for the lower concentrations of C18:0 in the bloodstream of microalgae-fed animals in the present study. Furthermore, PUFAs inhibit the growth of some bacterial species in the rumen that are responsible for biohydrogenation, including *Butyrivibrio proteoclasticus* (Jenkins et al., 2008). The decreased concentration of C18:1 observed in microalgae-fed steers in the present study may also be due to a decrease in the extent of lipolysis and biohydrogenation in the rumen. Such a decrease in lipolytic activity would allow for greater flow of biohydrogenation intermediates into the small intestine as described by Hess et al. (2008).

In the present study, dietary microalgae produced a 5.1-fold increase ($P < 0.0001$) in plasma n-3 fatty acids and decreased ($P < 0.01$) plasma n-6 fatty acids compared to control. As a result, the n-3:n-6 ratio in plasma from steers fed microalgae was 6.7-fold greater than plasma from steers fed the control diet ($P < 0.001$). All n-3 fatty acids were increased in plasma of steers supplemented with microalgae ($P < 0.01$) with 18:3n-3 increasing 1.2-fold, 20:3n-3 increasing 18-fold, 20:5 increasing 19.7-fold, and 22:6 increasing 7.5-fold. The n-6 fatty acids that decreased in plasma at slaughter with microalgae supplementation were 18:3n-6 and 20:3n-6 ($P \leq 0.01$). Previous studies have demonstrated that fish oil increases duodenal flow of n-3 fatty acids (Lee et al., 2005, 2008; Kim et al., 2008; Duckett and Gillis, 2010; Shingfield et al., 2010). The higher circulating levels of n-3 fatty acids observed for animals fed microalgae in the current study indicates

that dietary microalgae may have decreased ruminal biohydrogenation and resulted in a greater post ruminal flow of unsaturated and n-3 fatty acids.

Saturated fatty acid content of the human diet continues to be a concern for health-conscious consumers. A low ratio of PUFA to SFA (P:S ratio), as found in beef, increases the risk of cardiovascular and other diseases. The recommended P:S ratio in a normal human diet is 0.4 (Department of Health, 1994); however, beef typically has a ratio of 0.1 (Enser et al., 1996). Fatty acid composition of the LM in the present study is presented in Table 4. Dietary microalgae increased MCFA and decreased LCFA ($P = 0.02$) in the LM. Specifically, microalgae increased C14:0, C15:0, and C16:0 ($P \leq 0.03$) and decreased C18:1, C18:3n-6, and 20:3n-6 ($P \leq 0.02$). Dietary microalgae tended to decrease MUFA ($P = 0.06$), but had no effect on SFA or PUFA content ($P = 0.14$). However, microalgae supplementation increased LM concentration of *cis*-9, *trans*-11 conjugated linoleic acid ($P < 0.001$). The majority of consumed PUFA is biohydrogenated in the rumen and is not incorporated into muscle intact (Jenkins et al., 2008). However, Cooper et al. (2004) observed that PUFA from microalgae are less biohydrogenated compared to fish oil and in fact, supplemental microalgae has increased PUFA and DHA content of ruminant meat (Nute et al., 2007; Sampels et al., 2010; Meale et al., 2014) and milk (Boeckaert, et al., 2008; Abughazaleh et al., 2009; Glover et al., 2012; Stamey et al., 2012; Vahmani et al., 2013). It is thought that the n-3 long-chain PUFA inhibit the final step of ruminal biohydrogenation where *trans*-11 18:1 is converted to 18:0 (AbuGhazaleh and Jenkins, 2004). The enhanced ruminal outflow of *trans*-11 18:1 increases the tissue content of *trans*-11 18:1 as well as that of CLA, because of tissue conversion of *trans*-11 18:1 to CLA (Bauman and Griinari, 2003). Desaturation of *trans*-11 18:1 into CLA in tissues is the major source of CLA in tissues (Bauman and Griinari, 2003). Increased *trans*-11 18:1 and CLA in milk and beef tissue is desirable, because of their reported health benefits for humans (Lock and Bauman, 2004).

Microalgae more than doubled the n-3 fatty acid content of beef ($P < 0.0001$) and had no effect on n-6 content ($P = 0.67$). As a result, the n-3:n-6 ratio more than doubled ($P < 0.0001$) because of microalgae supplementation. This increase in n-3 content of beef is similar to that observed for plasma n-3 fatty acids. The primary n-3 fatty acids in muscle impacted by microalgae supplementation in this study were C20:5, which was increased 4-fold ($P < 0.0001$) and C22:6, which was increased 6.25-fold ($P < 0.0001$).

Table 4. Effect of microalgae on fatty acid composition of the LM

Fatty acid ³	Treatment ^{1,2}		SEM	P value
	Control	Microalgae		
C10:0	0.01	0.01	0.00	0.26
C12:0	0.01	0.01	0.00	0.26
C14:0	2.97	3.25	0.08	0.02
C14:1	0.68	0.73	0.02	0.19
C15:0	0.33	0.37	0.01	0.03
C16:0	26.78	27.76	0.27	0.01
C16:1 <i>cis</i> -9	3.59	3.61	0.15	0.93
C17:0	0.49	0.54	0.04	0.32
C17:1	2.09	1.89	0.31	0.65
C18:0	12.92	12.76	0.46	0.80
C18:1 <i>cis</i> -9	42.65	40.82	0.49	0.01
C18:2	4.09	4.10	0.14	0.97
CLA <i>cis</i> -9, <i>trans</i> -11	0.50	0.61	0.02	<0.001
CLA <i>trans</i> -10, <i>cis</i> -12	0.03	0.04	0.00	0.39
C18:3n-3	0.06	0.07	0.01	0.50
C18:3n-6	0.37	0.31	0.02	0.01
C20:0	0.29	0.23	0.03	0.16
C20:2	0.20	0.20	0.02	0.98
C20:3n-3	0.02	0.03	0.01	0.38
C20:3n-6	0.21	0.17	0.01	0.02
C20:4	0.50	0.49	0.04	0.83
C20:5	0.05	0.20	0.01	<0.0001
C22:2	0.02	0.02	0.00	0.31
C22:6	0.04	0.25	0.01	<0.0001
MCFA ⁴	3.99	4.36	0.11	0.02
LCFA ⁵	96.01	95.64	0.11	0.02
Total SFA	43.80	44.93	0.53	0.14
Total MUFA	49.86	48.40	0.57	0.06
Total PUFA	6.24	6.67	0.20	0.14
PUFA:SFA	0.14	0.15	0.01	0.44
n-3 fatty acids	0.32	0.73	0.04	<0.0001
n-6 fatty acids	5.37	5.27	0.18	0.67
n-3:n-6	0.06	0.14	0.01	<0.0001

¹Treatments were 1) control: ground corn top-dress (454 g/steer daily) with no microalgae or 2) microalgae: ground corn top-dress (454 g/steer daily) with 100 g/steer daily as ForPLUS (Alltech Inc., Nicholasville, KY). The control top-dress contained 3 mg/d Se as sodium selenite to equalize Se concentrations.

²Six pens/treatment; five steers/pen.

³Molar percentage.

⁴Medium-chain fatty acids ($\leq 15:1$).

⁵Long-chain fatty acids ($\geq 16:0$).

Microalgae similarly increased n-3 content of lamb (Hopkins et al, 2014; Meale et al., 2014; Ponnampalam et al., 2016). In contrast, Stokes et al. (2016) observed that inclusion of deoiled algae meal + soybean hulls decreased n-3 fatty acid content of the LM. It may be that the de-oiling process in the algae meal used in Stokes et al. (2016) removes significant n-3 or the soybean hulls had a negative effect on n-3 fatty acid concentrations. The n-3:n-6 ratio is an important nutritional index affecting cardiovascular and other diseases and a ratio above 0.25 is considered optimal for human health (Simopoulos, 2008).

Eicosapentaenoic acid and DHA are the most potent anti-inflammatory n-3 fatty acids and would have the most beneficial effects on human health. Although microalgae increased EPA and DHA concentration, and the n-3:n-6 ratio of beef to 0.14 in the present study, an optimal EPA and DHA concentration and n-3:n-6 ratio was not achieved. Thus, it may be difficult for humans to consume enough beef from cattle fed microalgae to have a health impact.

Carcass characteristics and lipid oxidation are presented in Table 5. Microalgae did not affect carcass characteristics ($P \geq 0.42$) or muscle Se content

Table 5. Effect of microalgae on carcass characteristics of steers

Item	Treatment ^{1,2}		SEM	P value
	Control	Microalgae		
HCW, kg	397.2	396.0	3.72	0.83
Dressing percent, %	63.9	63.7	0.38	0.65
Fat thickness, cm	1.63	1.55	0.094	0.65
Rib-eye area, cm ²	84.5	85.8	1.16	0.42
KPH, %	1.98	1.95	0.033	0.54
Yield grade	3.62	3.48	0.121	0.42
Marbling score	431.7	443.3	18.30	0.66
Quality grade distribution				
Select, %	3.0	7.0	4.7	0.63
Choice ⁻ , %	42.0	30.0	6.4	0.23
Choice ^o , %	35.0	43.0	5.9	0.35
Choice ⁺ , %	10.0	7.0	4.7	0.63
Prime, %	10.0	13.0	6.2	0.72
TBARS, ³ mg malonaldehyde/kg				
2 d of aging	0.157	0.143	0.0878	0.91
21 d of aging	0.732	0.967	0.0878	0.08

¹Treatments were 1) control: ground corn top-dress (454 g/steer daily) with no microalgae or 2) microalgae: ground corn top-dress (454 g/steer daily) with 100 g/steer daily as ForPLUS (Alltech Inc., Nicholasville, KY). The control top-dress contained 3 mg/d Se as sodium selenite to equalize Se concentrations.

²Six pens/treatment; five steers/pen.

³Thiobarbituric acid reactive substances.

($P \geq 0.60$; data not shown). In contrast, [Meale et al. \(2014\)](#) observed improved muscling scores in lambs when microalgae was included up to 2% of the diet on a DM basis and [Stokes et al. \(2016\)](#) reported decreased fat thickness, KPH%, and yield grade with a deoiled microalgae meal-based product that was included in the diet up to 42%. Muscle tissue fatty acids affect several aspects of meat quality including the firmness of fat and color, lipid stability, and flavor of the meat ([Wood et al., 2004](#)). Increased unsaturation of fatty acids can lead to a reduced retail display shelf-life due to accelerated lipid and color oxidation. In the present study, microalgae supplementation did not affect lipid oxidation in meat aged 2 d ($P = 0.91$), but tended to increase TBARS after 21 d of aging ($P = 0.08$), indicating that over a longer period of time shelf stability of meat from cattle fed microalgae may be compromised. However, [Campo et al. \(2006\)](#) reported that the TBARS values less than 2 mg of malonaldehyde/kg were considered acceptable by a sensory panel, thus steaks from steers fed microalgae were within limits of acceptability. Moreover, [Robertson \(1992\)](#) observed that meat vacuum packed and refrigerated rather than displayed did not have problems with oxidation and remained in good condition for up to 6 wk.

In conclusion, feeding microalgae did not change growth or carcass characteristics of steers. However, microalgae improved G:F, increased days

on feed, and increased CLA, n-3 fatty acids, and the n-3:n-6 ratio in beef, which may potentially benefit human health but may decrease shelf life of steaks. In addition, steers fed microalgae secreted less insulin after a glucose infusion, resulting in less glucose clearance from the blood, which indicated that metabolism was altered under the experimental conditions of this study.

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