Research, Society and Development, v. 9, n. 7, e675974568, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i7.4568

Demeda, MA, Tomaluski, CR, Baggio, C, Mateus, K, Petrolli, TG, Mueller, LF, Pereira, ASC, Griebler, L & Zotti, CA. (2020). Feeding microalgae (*Schizochytrium limacinum*) to beef steers increases meat omega-3 content. ***Research, Society and Development***, 9(7): 1-18**,** e675974568.

Microalgas (*Schizochytrium limacinum*) para novilhos de corte aumentam o teor de ômega 3 da carne

Feeding microalgae (*Schizochytrium limacinum*) to beef steers increases meat omega-3 content

Microalgas (*Schizochytrium limacinum*) para novillos carniceros eleva el contenido de omega-3 en la carne

Recebido: 15/05/2020 | Revisado: 21/05/2020 | Aceito: 25/05/2020 | Publicado: 02/06/2020

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Resumo

Novas alternativas para aumentar o conteúdo de ácidos graxos poliinsaturados na carne são necessárias. Os suplementos alimentares como microalgas são uma estratégia para enriquecer a carne com ômega 3. O objetivo do estudo foi avaliar desempenho, perfil de ácidos graxos (AG) da carne e características de carcaça de novilhos alimentados com microalgas na dieta. Dezesseis novilhos (peso médio 299,6 ± 7,4 kg) foram distribuídos aleatoriamente em dois grupos: o grupo controle (CTL) e o grupo microalgas (ALG; 1.7% da matéria seca). A dieta (40% de silagem de milho e 60% de concentrado) foi oferecida duas vezes ao dia durante 84 dias. A suplementação de microalgas aumentou 4,44 vezes os ácidos graxos ômega-3 no músculo Longissimus thoracis e lombar, e reduziu 3,6 vezes a relação ômega 6:ômega-3. As concentrações de 20: 5n3 e 22: 6n3 aumentaram 7 e 20,5 vezes, respectivamente, com a adição de microalgas. Entretanto, as microalgas diminuíram 16,5% o consumo de matéria seca (P <0,0001), além de 19,1% o ganho médio diário (P = 0,035) e 5,5% a capacidade de retenção de água muscular (P = 0,02). O uso de microalgas reduz o desempenho, mas não altera as características da carcaça e apresenta potencial para melhorar o perfil de AG da série ômega 3. Os consumidores podem ser atraídos para aumentar a ingestão de gordura poliinsaturada ômega

3 da carne bovina. Esses resultados podem apoiar a decisão dos nutricionistas ou produtores no momento de utilizarem microalgas em bovinos de corte, desde que se apresente economicamente viável.

Palavras-chave: Ácidos graxos; Carcaça; Gado de corte.

Abstract

New alternatives to increase the content of polyunsaturated beef fat are necessary. Feed supplements like microalgae are a strategy to enrich meat with omega 3. The aim of the study was to evaluate growth performance, fatty acid profile of meat and carcass characteristics of steers fed with microalgae in the diet. Sixteen steers (mean weight 299.6 ± 7.4 kg, seven months) were randomly assigned to two groups: the control group (CTL) and the microalgae group (ALG; daily feeding of 1.7% of dry matter as microalgae). The diet (40% corn silage and 60% concentrate) was offered twice daily for 84 days. Microalgae supplementation was associated with a 4.44-fold increase in total omega-3 fatty acids in the longissimus thoracis and lumbar muscle, reflecting a 3.6-fold reduction in the omega-6: omega-3 ratio. The concentration of 20: 5n3 and 22: 6n3 increased by 7 and 20.5 times, respectively, with the addition of microalgae. However, microalgae decreased the consumption of dry matter (P <0.0001) by 16.5%, the average daily gain (P = 0.035) by 19.1% and the muscle water-holding capacity (P = 0.02) by 5.5% compared to control. The use of microalgae reduces growth performance, but does not alter the characteristics of the carcass and has the potential to improve the FA profile of the omega 3 series. Consumers may be attracted to increase their intake of polyunsaturated omega 3 fat from beef. These results can support the decision of nutritionists and farmers to use microalgae in beef cattle, as it becomes economically viable.

Keywords: Fatty acids; Carcass traits; Beef cattle.

Resumen

Nuevas opciones son necesarias para incremento de ácidos gordos polinsaturados en la carne. Los complementos alimenticios como las microalgas son una estrategia para enriquecer la carne con omega 3. Así, el objetivo del estudio fue evaluar el rendimiento, el perfil de ácidos gordos (AG) de las características de la carne y el canal de novillos suplementados con microalgas en la dieta. Dieciséis novillos (peso promedio 299.6 ± 7.4 kg) fueron repartidos aleatoriamente en dos grupos: el grupo control (CTL) y el grupo de microalgas (ALG; 1.7% de materia seca). La dieta (40% de ensilaje de maíz y 60% de concentrado) se ofreció dos veces al día por 84 días. La suplementación con microalgas aumentó 4,44 veces los ácidos gordos omega-3 en el músculo torácico y lumbar Longissimus, y redujo la relación

omega 6: omega-3 en 3,6 veces. Las concentraciones de 20: 5n3 y 22: 6n3 aumentaron 7 y 20.5 veces, respectivamente, con la adición de microalgas. Sin embargo, las microalgas disminuyeron el consumo de materia seca en un 16.5% (P <0.0001), el 19.1% de la ganancia diaria promedio (P = 0.035) y aún, el 5.5% de la capacidad de retención de agua muscular (P = 0.02). El empleo de microalgas reduce el rendimiento, pero no altera las características del canal y tiene el potencial de mejorar el perfil de AG de la serie omega 3. Los consumidores pueden sentirse atraídos por aumentar la ingesta de grasas poliinsaturadas omega 3 de carne bovina. Estos resultados pueden respaldar la decisión de los nutricionistas o productores cuando usan microalgas en el ganado vacuno, siempre que sea económicamente viable.

Palabras clave: Ácidos grasos; Canal; Ganado vacuno.

1. Introduction

Until recently, beef was considered to be harmful to human health because of its high content of saturated fatty acid (SFA), especially when compared to meat from fish and poultry (Ladeira e Oliveira, 2007). However, in recent years, the consumption of animal protein has been growing considerably; coincidentally, there has been an increase in the need to find adequate sources of protein for food production.

Physicians recommend daily intake of foods that are sources of polyunsaturated fatty acids, especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Their intake is important because of the roles they play in growth, development, and physiological function (Das, 2006) and for their role in suppressing or preventing inflammation associated with cancer, cardiovascular disease, and type 2 diabetes (Das, 2008, Azrad et al., 2013).

An alternative found to improve the quality of the products available to consumers is the use of microalgae in animal diets. Microalgae have been used in the animal feed industry since the 1970s; they are considered suitable alternatives for improving animal health, thereby improving the quality of their meat and meat products (Ścieszka and Klewicka, 2018).

Currently, the high protein content found in various microalgae species is one of the primary factors favoring their use in animal feed (Kovač et al., 2013). Heterotrophic microalgae (produced in bioreactors) are important sources of vitamins, minerals and mainly fatty acids, EPA and DHA, and there has been identification of their effects on the immune system (Fike et al., 2001), intestinal health (Dierick et al., 2010) an general growth rates (Fike et al., 2001; Clayton et al., 2014) in various species.

In addition to their role in animal health and performance, the ability of microalgae to enrich animal products with omega fatty acids (especially EPA and DHA) have been tested in many studies. In monogastric animals, microalgae supplementation for enrichment of meat and eggs has been used successfully (Moran et al., 2017; Fraeye et al., 2012). However, in ruminants, this process requires somewhat more attention, because extensive lipolysis and subsequent biohydrogenation of unsaturated fatty acids occurs in the rumen, presenting a challenge for metabolism of polyunsaturated fatty acids, as is the case for omega 3 fatty acids in products such as milk and meat (Jenkins et al., 2008, Shingfield et al., 2013).

Therefore, we hypothesized that animals receiving diets supplemented with microalgae will increase the polyunsaturated fatty acid (PUFA) profiles of their meat without altering performance and carcass traits. The aim of the study was to determine whether the addition of *Schizochytrium limacinum* microalgae alters growth performance, carcass traits and beef quality.

2. Materials and Methods

The study was conducted at the La Salle Agricultural College, located in Xanxerê, SC - Brazil. All activities were approved by the Committee on Ethics in the Use of Animals of University of Western of Santa Catarina (Protocol 03/2017).

Sixteen steers (F1 Angus x Nelore), seven months old, and average weight of 299.6 ± 7.4 kg were used. The animals were randomized into blocks according to initial body weight and were placed in individual pens (4.5 x 22.2 m) with an uncovered soil floor and provided with shade and with free access to water and feed.

Two treatments were tested: Control (CTL n = 8): fed basal diet without addition of microalgae; and Microalgae (ALG n = 8): fed 17 g/kg DM in the basal diet (Table 1).

Table 1. Ingredient proportion (% MS) and chemical composition of the diet fed control (CTL) or microalgae (ALG) to beef steers.

Ingredient	CTL	ALG
Corn silage [A]	40.62	38.9
Cracked corn	42.77	42.77
Soybean meal	14.0	14.0
Limestone	0.9	0.9
Urea	0.95	0.95
Sodium chloride	0.06	0.06
Mineral [B]	0.7	0.7
Microalgae	-	1.72
TDN	78.2	76.9
Crude protein	15.4	15.4

TDN, Total digestible nutrient. [A] Corn silage with 32.4% MS, mean particle size 12.6 mm e peNDF$_{8mm}$ 30.8%. [B] Mineral calcium 113 g/kg; Phosphorus 45 g/kg; Sulfur, 40 g/kg; Magnesium 44 g/kg; Potassium 61.5 g/kg; Sodium 114.5 g/kg; Cobalt, 48.5 mg/kg; Copper 516 mg/kg; Iodine 30 mg/kg; Manganese, 760 mg/kg; Selenium 9 mg/kg; Zinc 2,516.5 mg/kg; Fluoride 450 mg/kg.

The microalgae (*Schizochytrium limacinum*) had the following composition: DM (g/kg): 974 ± 1.0; fatty acids (g/100 g FA total): 33.2 ± 3.0; C16:0 52.58 ± 0.36; C22:5 cis-4, cis-7, cis-10, cis-13, cis-16: 6.31 ± 0.06; C22:6 cis-4, cis-7, cis-10, cis-13, cis-16, cis-19: 29.98 ± 0.28; ω-6: 6.56 ± 0.07; ω-3: 30.50 ± 0.29.

The diet was fed twice a day, in equal proportions, adjusting dry matter intake daily by weighing feed offered and refusals. Steers were weighed with 16-hour fast every 21 days. At the end of feedlot (84 days) steers were slaughtered after 16-hours of fasting at a commercial slaughterhouse located in Xanxerê, SC, Brazil (8 km of distance from the feedlot). The hot carcass weight (HCW) was obtained at slaughter and the dressing percentage was calculated after rigor mortis (24 h after slaughter). In addition, the subcutaneous fat thickness (SFT) and rib-eye area (REA) of the longissimus muscle were measured in the left carcass between the 12th and 13th ribs. Two samples were collected after transverse cutting in the carcass,

specifically in the longissimus dorsi muscle to determine the fatty acid profile and instrumental analysis.

The physicochemical quality of the meat was determined in the longissimus thoracis et lumborum (LTL) muscle. Weight loss upon defrosting was determined. The superficial coloration of the meat was measured at three different random points, after 30 seconds of exposure of samples to atmospheric oxygen. Color was measured using a Konica Minolta Colorimeter (CR 400) to obtain the coordinates L, a*, and b* in the Cielab system.

The water holding capacity (WHC) was calculated using the filter paper pressure method (Hamm, 1986), performed after weighing 5 grams of meat from sub-samples extracted from the Longissimus thoracis et lumborum muscle without ribs or aponeuroses. Subsequently, the samples were placed between standard filter papers and compression at 2.250 Kg was performed for 5 minutes, followed by re-weighing.

Cooking loss was measured after the initial weighing and the final weighing of the steaks obtained from the the Longissimus thoracis et lumborum muscle. These were roasted on an electric grill at an internal temperature of 72 ºC. After cooking, the samples were maintained at room temperature (21ºC) and then seven cylinder samples (1.25 cm diameter) were taken parallel to the fiber direction of each roasted sample and analyzed using the Warner-Bratzler shear force (WBSF) method as described by Wheeler et al. (2001). The instrument was brought into contact with the sample at a test speed of 10 mm/s, after which the test speed was reduced to 5 mm/s. The measured parameter was total force divided by the total sample area (Kgf/cm²).

The fatty acid profile of meat (FAs) was performed at the Meat Science Laboratory in the Department of Animal Nutrition and Production at University of Sao Paulo, Pirassununga/SP – Brazil. FAs were extracted from intramuscular fat of the LTL muscle as described by Folch et al. (1957) while methyl esters were formed according to Kramer et al. (1997). The FAs were quantified by gas chromatography (GC-2010 Plus AOC 20i auto-injector; Shimadzu, Kyoto, Japan) with a SP-2560 capillary column (100 m x 0.25 mm I.D. x 0.02 mm; Supelco, Bellefonte, PA). The initial temperature of 70°C was gradually increased to 175 °C (13°C/min) and maintained for 27 min, with a further increased to 215 °C (4°C/min) and maintained for 31 min, using hydrogen as carrier gas flowing at 40 cm3/s. FAs were identified by comparing the retention time of sample methyl esters with the FAs standard C4-C24 (F.A.M.E mix Sigma®) and GLC 463 Reference Mixture Nu Check, vaccenic acid C18:1 trans-11 (V038-1G, Sigma®) C18:2 trans-10 cis-12 (UC-61M 100 mg), CLA and C18:2 cis-9, trans-11 (UC- 60M 100 mg), (Sigma®), tricosanoic acid (Sigma®), and nonadecanoic acid (Sigma®). FAs were quantified by normalizing the area under the curve of methyl esters using

the GS 2.42 software. FAs contents were expressed as percentage of total FA methyl ester quantified.

Data were analyzed from a randomized block design using PROC MIXED (SAS version 9.1) and the following model: Yij = μ + Bi + Tj + eij, where Yij = the response variable of interest, μ = the overall mean, Bi = the random effect of the blocks (initial weight; 1 or 2), Tj = the fixed effect of treatment (j = CTL and ALG) and eij = the residual error. Each steer was considered as an experimental unit. Means were considered statistically significant when $P \leq 0.05$.

3. Results

Steers receiving microalgae had lower DMI (P <0.0001) and lower average daily gain (ADG) (P = 0.0349), with no differences in the final body weight (P = 0.305) or feed efficiency (P = 0.625) (Table 2). The carcass traits, HCW (P = 0.409), DP (P = 0.569), REA (P = 0.86) and SFT (P = 0.941) were not affected by treatments. Similar results in DMI were obtained by De la Fuente et al. (2014) using fish oil supplementation and by Burnett et al. (2012), Borghi (2018) and Urrutia et al. (2016) using microalgae supplementation. This reduction might be attributed to the lower palatability of the diet by animals because of the characteristic odor of the seaweed after mixing (Díaz et al., 2017).

Table 2. Performance and carcass traits of steers fed control (CTL) or microalgae (ALG) diet for 84 days.

Variables	CTL	ALG	SEM	*P*-value
DMI (kg.day)	9.26	7.73	0.19	<0.0001
ADG (kg.day)	1.41	1.14	0.06	0.035
Final BW (kg)	415.8	394.2	10.2	0.305
Gain:Feed	0.153	0.146	0.006	0.625
HCW (kg)	221.87	212	5.75	0.409
DP (%)	53.32	53.78	0.39	0.569
REA (cm^2)	83.03	83.94	2.46	0.860
SFT (mm)	3.87	3.81	0.39	0.941

DMI, dry matter intake; ADG, average daily gain; HCW, hot carcass weight; DP, dressing percentage; REA, Rib eye area; SFT, Subcutaneous fat thickness; SEM, Standard error of mean.

The reduction of DMI may be the main justification for the lower ADG observed, however this finding did not impact carcass traits as observed by Carvalho *et al.* (2018). The lower ADG resulted in an increase in days on feed (Borghi 2018), which is undesirable in the production chain owing to the higher costs. Then feedlot diets with considerable amount of microalgae need to be carefully recommended. The divergences in the results from our study compared to others may have been caused by several factors, including composition of the diet offered, quantity of microalgae used, efficiency in the mixing and acceptance on the part of the animals.

Supplementation with microalgae reduced WHC (P = 0.02) and increased color L* (P = 0.01), a* (P = 0.04), b* (P = 0.03) and chroma (P = 0.03) and showed a tendency to increase º hue (P = 0.06) (Table 3). However, thawing loss (P = 0.79) and cooking losses (P = 0.76) were not affected by treatments.

The lower WHC implies loss of nutritive value due to the released exudates, resulting in drier meat with less tenderness (Pardi *et al.*, 2001). The WHC interferes on softness characteristics such as firmness and tactile sensation; however, the shear force (P = 0.50; Table 3) was not different between the treatments, in agreement with the findings of Phelps *et al.* (2016).

Table 3. Physico-chemical properties of Longissimus dorsi from steers fed control (CTL) or microalgae (ALG) diet.

Variables	CTL	ALG	SEM	*P*-value
L*	36.1	39.3	0.57	0.01
a*	14.5	15.6	0.30	0.04
b*	7.48	8.73	0.29	0.03
Chroma	16.3	17.9	0.39	0.03
ºHue	26.8	28.9	0.55	0.06
Thawing loss (%)	3.76	4.12	0.24	0.79
WHC (%)	82.0	77.5	0.05	0.02
Cooking loss (%)	23.5	24.2	1.08	0.76
Shear force (Kgf.cm²)	3.52	3.66	0.10	0.50

L*., lightness; a*., redness; b*., yellowness; WHC., water holding capacity; SEM: Standard Error of the Mean.

In the present study the values of L*, a* and b* were close to the values reported by Muchenjea *et al.* (2009), as well as to values reported by Abularach *et al.* (1998). The control group presented meat with a low intensity of red, whereas the microalgae group presented meat with high luminosity and yellow content. These results may be associated with the lower WHC found in this study. Andrade *et al.* (2010) reported that higher luminosity meat surfaces is correlated with lower water retention capacity and higher red and yellow intensities.

The increase in chroma values (P = 0.03) and the trend of color increase observed in this study may be explained by the discoloration of meat over time according to Lee *et al.* (2005). In the study of Phelps *et al.* (2016), surface color measurements followed the typical patterns associated with steak discoloration; nevertheless, heifers fed microalgae heifers showed color reduction. This is explained by the decrease in the percentage of superficial oxymyoglobin and the simultaneous increase in the percentage of metmyoglobin as concentration of fed microalgae as maturation time increased.

The increase in coloration observed in the current trial for the microalgae treatment was not expected, because the ingestion of higher amounts of polyunsaturated fatty acids via diet resulted in an increase in long chain fatty acids in the meat (Table 4). As already shown, beef with higher levels of polyunsaturated acids is more susceptible to lipid oxidation (Ladeira e Oliveira, 2007), which in turn has a strong relationship with oxidation of myoglobin, conferring a reduction in meat color (Faustman and Cassens, 1990).

Darker meats have lower market value because they are rejected by the consumer, exacerbating the risk of deterioration. The change from red to dark red meat is caused by the transformation of oxymyglobin to metoxymyoglobin, which occurs in parallel to the spoilage process, diminishing palatability (Wood *et al.*, 2003).

Beef from steers or even older animals with pale pink coloring is also rejected by consumers, as they prefer meat with bright red colors, characteristic of fresh, healthy meat. Fernandes *et al.* (2008) working with Canchim heifers in confinement receiving sunflower seeds, reported the following for meat color: 37.39 (L*) and 15.95 (a*).

3.1 Fatty acid profile

There was a 4.4-fold increase (P = 0.0063) in the total fatty acids of the omega-3 series in the *longissimus dorsi*, reflecting a 3.6-fold reduction (P < 0.0001) in the omega6:omega3 ratio when the microalgae were added to the diet. Concentrations of EPA (20:5 n3) and DHA

(22: 6 n3) increased 7 and 20.5 times, respectively, with addition of microalgae (Table 4). Also, changes (P <0.05) were observed in levels of C18:0, C18:1-cis fatty acids and their trans-8, trans-9, trans-10 and trans-11 isomers, showing a decrease of the fatty acid content in the cis form and increase in the levels of the molecules in the trans forms. Lower beef meat n-3 enrichment were described by Carvalho *et al.* (2018), who reported 4 and 6.25-fold of 20:5 n-3 and 22:6 n-3 respectively when microalgae was used.

There was a reduction in levels of fatty acid C20:0 (P = 0.015) and increase (P >0.05) in levels of fatty acids C20:4, C20:5, C22:1, C22:4 and C22:6 in the meat. There were no effects (P >0.05) on the levels of the other fatty acids analyzed, with no differences (P >0.05) on total saturated fatty acids (SFA), total mono-unsaturated fatty acids (MUFA), total polyunsaturated fatty acids (PUFA), PUFA:SFA ratio, or total PUFA levels.

Table 4. Fatty acid profile of Longissimus dorsi of steer fed control (CTL) or microalgae (ALG) diet.

Trait[1]	CTL	ALG	SEM	*P*-value
10:0	0.051	0.055	0.003	0.557
12:0	0.064	0.071	0.004	0.445
13:0	0.015	0.019	0.001	0.335
14:0	2.501	2.643	0.179	0.706
14:1	0.464	0.654	0.0081	0.251
15:0	0.334	0.379	0.021	0.321
15:1	0.077	0.124	0.013	0.079
16:0	24.25	25.85	0.624	0.209
16:1 *cis* 7	0.128	0.161	0.028	0.596
16:1 *cis* 9	2.959	3.4	0.268	0.429
16:1 *trans* 13	0.293	0.185	0.031	0.091
16:1 *trans* 9	0.296	0.377	0.024	0.093
17:0	0.866	0.844	0.041	0.799
17:1 *cis* 10	0.646	0.767	0.052	0.258
18:0	16.013	11.364	0.761	0.0003
18:1 *cis* 11	1.995	1.944	0.051	0.635
18:1 *cis* 6/8	0.323	0.639	0.053	0.0006
18:1 n-9 *cis*	33.06	25.214	1.277	0.0002
18:1 *trans* 10	0.676	2.867	0.355	0.0002
18:1 *trans* 11	1.036	1.744	0.155	0.017
18:1 *trans* 6/8	0.167	0.338	0.034	0.0085
18:1 *trans* 9	0.221	0.574	0.064	0.0022
18:2 n-6 *cis*	5.125	5.608	0.552	0.677
18:2 n-6 *trans*	0.099	0.147	0.011	0.016
18:3 n-3	0.22	0.357	0.042	0.106
18:3 n-6	0.034	0.044	0.005	0.424
19:0	0.085	0.118	0.011	0.117
19:1 *cis* 7	0.031	0.063	0.011	0.136

19:1 *cis* 11/13	0.086	0.114	0.012	0.249
20:0	0.092	0.065	0.005	0.015
20:1 *cis* 11	0.192	0.122	0.027	0.225
20:1 *cis* 8	0.056	0.049	0.004	0.452
20:2 n-6	0.056	0.062	0.018	0.884
20:3 n-6 *cis* 8	0.277	0.419	0.053	0.191
20:3 n-9	0.111	0.114	0.015	0.912
20:4 n-3	0.0271	0.2726	0.049	0.007
20:4 n-6	1.275	1.745	0.288	0.436
20:5 n-3	0.212	1.489	0.251	0.0056
21:0	0.015	0.02	0.002	0.239
22:0	0.026	0.032	0.005	0.605
22:1 n-9	0.011	0.043	0.007	0.041
22:2 n-6	0.012	0.045	0.009	0.232
22:4 n-6	0.081	0.172	0.022	0.034
22:5 n-3	0.405	0.729	0.104	0.124
22:5 n-6	0.0704	0.198	0.037	0.1004
22:6 n-3	0.054	1.11	0.0202	0.0037
23:0	0.035	0.0607	0.007	0.064
CLA *cis* 9 *trans* 11	0.347	0.457	0.048	0.269
CLA *cis* 9 *trans 7*	0.048	0.0649	0.006	0.215
Total SFA[2]	44.377	41.496	0.926	0.123
Total MUFA[3]	42.557	39.308	1.259	0.207
Total PUFA[4]	8.41	12.91	1.415	0.115
Total ω-6 PUFA[5]	7.01	8.06	0.892	0.576
Total ω-3 PUFA[6]	0.951	4.211	0.646	0.0063
PUFA:SFA ratio	0.191	0.328	0.042	0.114
ω-6:ω-3 ratio	8.08	2.25	0.898	<0.0001

CLA., conjugated linoleic acid; SFA., saturated fatty acids; MUFA., monounsaturated fatty acids; PUFA., total polyunsaturated fatty acids

[1] FAs expressed as a percentage of the total fatty acid methyl esters (FAME).

[2]Total SFA = 10:00 + 12:0 + 13:0 + 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 19:0 + 20:0 21:0 + 22:0 + 23:0 + 24:0.

[3]Total MUFA = 14:1 + 15:1 + 16:1 cis-7 + 16:1 cis-9 + 16:1 trans-13 + 16:1 trans-9 + 17:1 cis-10 + 18:1 cis-11 + 18:1 cis-6-8 + 18:1 n-9 cis + 18:1 trans-10 + 18:1 trans-11 + 18:1 trans-6-8 + 18:1 trans-9 + 19:1 cis-7 + 19:1 cis-11-13 + 20:1 cis-11 + 20:1 cis-8 + 22:1 n-9.

[4]Total PUFA = 18:2n-6 cis + 18:2n-6 trans + 18:3n-6 + 18:3n-3 + 20:2n-6 + 20:3n-3 + 20:3 n-6 cis + 20:3 n-9 + 20:4n-3 + 20:4n-6 + 20:5n-3 + 22:2n-6 + 22:3n-3 + 22:4n-6 + 22:5n-3 + 22:5n-6 + 22:6n-3 + CLA cis-9, trans-11 + CLA trans-7, cis-9.

[5]Total omega-6 PUFA = 18:2n-6 cis + 18:2n-6 trans + 18:3n-6 cis + 20:3 n-6 cis-8 + 20:4 n-6 + 22:2 n-6 + 22:4 n-6 + 22:5 n-6.

[6]Total omega-3 PUFA = 18:3 n-3 + 20:4 n-3 + 20:5 n-3 + 22:5 n-3 + 22:6 n-3.

The inclusion of marine algae in the diets caused changes in the activity of ruminal biohydrogenating bacteria, increasing the formation of trans-C18:1 fatty acids. As the unsaturated moieties exert toxic effects on ruminal bacteria, microalgae are rich in unsaturated fatty acids, there is a normal need for ruminal biohydrogenation. However, Altomonte *et al.* (2018) reported that there were

changes in beta-hydroxybutyrate pathway formation, associated with changes in the population of the bacterium *Butyrivibrio fibrisolvens*. As a result, there was a greater presence of trans fatty acids in the meat resulting from the partial biohydrogenation of these molecules in the rumen. Tsiplakou *et al.* (2017) observed the same when adding seaweed from the diet of goats, reporting reductions in the population of this bacterial species in the ruminal liquid. This factor may be a negative finding against the use of marine algae in animal diets, because the presence of trans fatty acids in foods of animal origin is associated with several metabolic dysfunctions, primarily associated with metabolism as well as fat and cholesterol transport.

However, it was possible to enrich the omega-3 series FA profile (C22:4 and C22:6), providing a more enriched and nutritionally appealing food for the consumer. Our data agree with those of Meale *et al.* (2014) and Carvalho *et al.* (2018), who observed increased levels of omega-3 fatty acids mainly docosahexaenoic acid, in the meat of animals supplemented with the same microalgae that we studied.

The higher levels of PUFAs in meat may give rise to other problems, primarily associated with oxidative stability, coloring and sensory traits of meat. Díaz *et al.* (2017) reported greater difficulty in maintaining the shelf-life of PUFA-enriched meats, while Urrutia *et al.* (2016) found lower consumer preference because of sensorial traits, that had been altered by microalgae supplementation in the animals' diets. By contrast, the inclusion of seaweed in animal feed can enrich the meat with other molecules beneficial to human health, also compensating for the low stability of these molecules.

5. Final Considerations

The use of dietary supplementation with microalgae reduced steer growth performance but did not alter carcass traits and offered potential to improve omega 3 series FA profiles. In addition, consumers may be attracted to increase their intake of polyunsaturated omega 3 fat from beef. These results can support the decision of nutritionists and farmers to use microalgae in beef cattle, as it becomes economically viable.

Conflict of Interest

There is no perceived conflict of interest.

Financial Support and Acknowledgments

We thank to the Programa de Mestrado em Sanidade e Produção Animal da Unoesc and Alltech for donating the product for research.

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