Feeding microalgae at a high level to finishing heifers increases the long-chain n-3 fatty acid composition of beef with only small effects on the sensory quality

by Rodriguez-Herrera, M., Khatri, Y., Marsh, S. P., Posri, W. and Sinclair, L. A.

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Feeding microalgae at a high level to finishing heifers increases the long chain n-3 fatty acid composition of beef with only small effects on the sensory quality.

Maricruz Rodriguez-Herrera[1], Yunus Khatri[1] , Simon P. Marsh[2] , Wilatsana Posri[1] & Liam A. Sinclair[2]

[1]Department of Food Science and Agri-Food Supply Chain Management, Harper Adams University, Edgmond, Newport, Shropshire TF10 8NB, UK.

[2]Department of Animal Production, Welfare and Veterinary Sciences, Harper Adams University, Edgmond, Newport, Shropshire TF10 8NB, UK.

Corresponding author: lsinclair@harper-adams.ac.uk

Running head: Microalgae and beef quality

Summary The aim of the study was to determine the effect of feeding a low and high level of microalgae (MA, high in C22:6n−3) on the fatty acids (FA) composition and sensory attributes of beef. Thirty Charolais cross Limousin/Friesian heifers were fed one of three diets (n= 10 per treatment): Control (no MA), low MA (LMA; inclusion rate of 15 g/kg) or high MA (HMA; inclusion rate of 30 g/kg) for 95 days before slaughter. Heifers fed HMA had a higher (P < 0.05) content of C20:5n−3: eicosapentaenoic acid (EPA), and C22:6n−3: docosahexaenoic acid (DHA) in the *longissimus thoracis* muscle than those receiving the Control (mean values for EPA of 0.5, 0.92, 1.20 and DHA of 0.31, 0.89 and 1.05 g/100g FA for Control, LMA and HMA respectively), and a lower n-3 to n-6 ratio (2.9, 1.9 and 1.6 in Control, LMA and HMA respectively; P < 0.001). Steaks from animals fed either of the MA diets had a marginally higher (P < 0.05) “seaweedy flavour” that was positively correlated to muscle C22:6n-3 concentration. Steaks from animals fed HMA were rated as being higher (P < 0.05) in tenderness, and had a lower (P < 0.05) shear force than those from Control fed animals. It is concluded that feeding microalgae at high levels can beneficially improve the health attributes of beef with only a few effects on sensory quality.

Keywords: Beef, fatty acid, muscle tissue, sensory evaluation.

Introduction

The very long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3) have been shown to have benefits in the modulation and prevention of coronary heart disease, in the early development and maintenance of retinal and brain tissues, and in the prevention of certain cancers (Abedi & Sahari, 2014; Zárate *et al.,* 2017). Oil-rich fish and fish oil have traditionally been the main sources of LC n-3 PUFA in the human diet (Welch *et al.,* 2002). However, the consumption of fish varies regionally, and in many western countries such as the UK are in decline (Welch *et al.,* 2002). The current intake of EPA + DHA in the UK is approximately 100 mg/day, which is considerably lower than the recommended value of 250-450 mg/day (SACN & COT, 2004; EFSA, 2010). Alternative dietary sources of these fatty acids (FA) such as meat based foods is therefore of considerable interest.

Beef has long been recognized as a valuable source of EPA and DHA in the human diet (Wood *et al.,* 2008). It has been proposed that the levels of LC n-3 PUFA in beef can be increased further by feeding dietary sources rich in those FA (Gibbs *et al.,* 2010). Feeding microalgae (MA) has been proposed as a means to improve the EPA and DHA content of beef, mainly because it is a rich source of LC n-3 PUFA (Givens *et al.,* 2006; Zárate *et al.,* 2016), and the degree of ruminal biohydrogenation has been reported to be lower than in other sources such as fish oil (Sinclair *et al.,* 2005). Previous studies have reported an increase in muscle deposition of LC n-3 PUFA following feeding of MA to lambs (Cooper *et al.,* 2004; Hopkins *et al.,* 2014; Meale *et al.,* 2014). However, few studies have evaluated the effect of feeding MA to cattle, particularly at high rates of inclusion, in concentrate finishing systems or when slaughtered at a low body fat content (Phelps *et al.,* 2016a,b).

Modifying the FA composition of muscle may influence certain characteristics of meat quality, including flavour, texture and lipid stability (Wood *et al.,* 2008). Highly

unsaturated FA such as LC n-3 PUFA are more susceptible to oxidation during retail and cooking, and high concentrations of these FA in the muscle can contribute to the development of undesirable off-flavours in cooked meat (Nute *et al.,* 2007). Lipid oxidation is recognised as one of the main causes of the deterioration of meat quality, therefore one of the main challenges to studies attempting to increase the concentration of LC n-3 PUFA in meat is to avoid oxidation and maintain the eating quality. However, few studies have been conducted to determine the effect of the dietary inclusion of MA on the sensory attributes of beef and the relationship with meat FA content, particularly when fed at high levels. The objectives of this study were to determine the effects of a low and high inclusion rate of MA (rich in DHA) in the diet of finishing heifers on the FA composition of intramuscular fat and subcutaneous adipose tissue, and the sensory attributes of beef.

Materials and method

Diets, animals and experimental procedure

Thirty Charolais cross Limousin/Friesian heifers with an initial mean age of 24 ± 0.8 months and live weight of 509 ± 40.0 kg were used. Prior to the study the animals were fed medium quality grass silage and were gradually adapted to a cereal based diet over a period of 17-d. After this period the animals were blocked according to live weight and randomly allocated within block to one of the three experimental diets, resulting in ten animals per diet. Three rates of MA (*Schizochytrium limacinum*; Alltech UK Ltd., Lincs, UK*)* were fed; Control (no MA), low MA (LMA; 15 g/kg dietary inclusion of MA) and high MA (HMA; 30 g/kg dietary inclusion of MA). The Control diet contained (g/kg) 750 barley, 100 dried, molassed sugar beet pulp, 75 soyabean meal, 50 molasses and 25 minerals and vitamins, with the proportional replacement of barley with MA in diets LMA and HMA. The MA was in the form of a dried powder with a lipid content of 580 g/kg dry matter (DM), and contained an EPA and DHA content of 0.28 and 27.2 g/100g FA respectively.

The diets were formulated to have a similar energy content and to be isonitrogenous, with vitamin E added as an antioxidant at 250 mg/kg.

Treatment diets were offered *ad libitum* via hoppers for 95 days with free access to wheat straw. Animals were double weighed at the beginning and end of the study using electronic weigh cells (Tru-Test, Auckland, New Zealand). Following slaughter in a commercial abattoir (ABP, Shrewsbury, UK) using a captive bolt and severing the arteries, the carcasses were hung for 48 h at 4°C, weighed and scored for conformation and fat class using a 15-point scale based on RPA (2011), where 1 = lowest and 15 = highest. Samples from the *longissimus thoracis* muscle and the subcutaneous adipose tissue (at the 10-12th rib) were then dissected vacuum-packaged and stored at -20°C prior to FA analysis, or conditioned at 1°C for 10 d and then stored at -20°C prior to sensory analysis, as described by Vatansever *et al.* (2000) and Nute *et al.* (2007).

Fatty acid analysis

The FA content of muscle and subcutaneous adipose tissue of all animals (n = 10 per treatment) was conducted according to the method described by O'Fallon *et al.* (2007) using C13:0 as an internal standard. Following methylation the FA's were analysed on a gas chromatograph (HP 6890, Germany) equipped with an auto sampler (Agilent 6890 injector, UK), a split injector and a flame-ionization detector (Agilent Inc. Wilmington, DE). The separations were performed on a CP-SIL 88 for FAME capillary column, 100 m length x 0.25 mm internal diameter and 0.2 µm film thickness (Agilent J&W, GC columns, UK). Oven temperature was first held at 70 °C for 2 min, then increased by 8 °C/min to 100 °C; 5 °C/min to 170 °C and finally 4 °C/min to 225 °C; this temperature was maintained until all peaks were analysed. The split ratio was 100:1. Hydrogen was used as the carrier gas and peaks were identified by comparison of retention times with a standard mixture (Supelco® 37 Component FAME Mix, Sigma-Aldrich, Dorset, UK). The area for each FA was adjusted using the recovery factors from a standard Supelco® 37

mixture, and normalized to 100%. Total FA content was quantified by reference to the area of the internal standard. The fatty acids were expressed as % of total fatty acids in intramuscular fat and subcutaneous adipose tissue, or mg/100 g fresh tissue (Cooper *et al.,* 2004; Nute *et al.,* 2007; Wachira *et al.,* 2004). Feed samples were bulked within month (resulting in n = 3 per treatment) and their FA composition determined by the method of Jenkins (2010; Table S1). Feed samples were also analysed according to AOAC (2012) for DM (method 934.01), ash (942.05), crude protein (CP; 990.03 using a Leco® FP-528 analyzer, LECO Corporation, USA), and neutral detergent fibre (NDF; Van Soest *et al.,* 1991).

Sensory assessment and shear force

The steaks were thawed overnight at 4 °C and then cooked using an auto-controlled roasting/baking oven (Self-Cooking Center® SCC 101, Rational AG, Germany). The samples were cooked, turning over half way through, and the internal temperature verified with a thermocouple probe (Therma 20, ETL Ltd, UK) inserted into the centre of each steak to ensure that it reached at least 73°C.

A descriptive sensory analysis was performed by 10 trained panellists. Following recruitment, the assessors were screened on the basis of their sensitivity to five basic tastes, ability to detect specific odours, numerical competence with respect to scaling and descriptive ability (BSI, 2014). The panellists were trained and calibrated for beef profiling in accordance with the guidelines of BS EN ISO 8586:2014 (BSI, 2014) and Gomes *et al.* (2014). During the training stage the panellists followed seven training sessions of two hours each with an open discussion to generate the lexicon to describe the beef steaks in terms of odour, taste, texture and after taste. The standard references for key sensory attributes were provided (adapted from Adhikari *et al.,* 2011, and Maughan *et al.,* 2012). There were also ballot training sessions conducted on the consensus lexicon, rating scale used (unstructured 15 cm line scale with two anchor

words) and intensity calibration using standard references with an interactive feedback screen using Compusense® five (Compusense., Guelph, Ontario, Canada). The performances of individual panellists and the panel were tested for their reliability and validity in order to retain, adjust and calibrate the measurements. The sensory profiles of all treatments (3) and replicates of treatments (10) were evaluated on the consensus ballot based on 2 test replications, resulting in the profiling of 60 meat samples. The sensory evaluation took place over 5 consecutive days, with the first three sessions allocated for the first test replication and the last three for the second. A Williams cross-over design was applied within each test replication to randomly balance first-order and carry-over effects. The evaluation sessions took place in individual booths equipped with Compusense computerised software. Five 1.5 cm steak cubes of each sample were served at room temperature and presented in a random order.

Shear force of the cooked steaks was measured based on the method described by Peachey *et al.* (2002), using a TA.HD Plus Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK), fitted with a Warner Bratzler blade with a rectangular slot and 30 kg load cell. Five 10 mm cubes from each sample were assessed. The cubes were sheared perpendicular to the fibre orientation with 2.0 mm/s crosshead speed. The resistance of the meat to shearing was recorded every 0.005 s and a curve (time vs. force) plotted. The parameters recorded were “Maximum shear force” (N), indicating the maximum force required to cut through the sample and “Work of shear” (N.s), which relates to the total work done to cut the sample, also referred as “toughness”.

Statistical analysis

The FA content of tissues, sensory analysis, texture of cooked steaks and animal performance were analyzed using ANOVA as a randomized complete block design. The statistical model was $yij = bi + tj + eij$, where b = blocks and t = treatments. Daily live weight gain was calculated as the final weight minus the initial weight divided by the

number of days on study. The results are presented as means for the treatments with s.e.d., and post-hoc analysis using Tukey's test at a 5% significance level. Pearson correlation coefficients were generated to describe the association between sensory scores and the percentage of n-3 PUFA in muscle, along with linear regression to obtain the significance values for the model. All analysis was conducted using Genstat (16th edition; VSN International Ltd., Oxford, UK).

Results and discussion

Fatty acid content of the *longissimus thoracis* muscle

The proportion of C22:6n-3 in the *longissimus thoracis* muscle was highest (P < 0.05) in cattle receiving the HMA diet and lowest in those receiving the Control diet, with those receiving LMA being intermediate (Table 1), with values comparable to other studies that have fed MA to sheep or cattle (Hopkins *et al.,* 2004; Phelps *et al.,* 2016a). The muscle from heifers fed the HMA diet also had the highest (P < 0.05) proportion of C20:5n-3, followed by LMA, with those receiving the Control diet the lowest. By contrast, the proportion of C22:5n-3 decreased (P < 0.05) with the inclusion of MA in the diet. The highest (P < 0.05) proportion of eicosatrienoic (C20:3n-6) and docosatetraenoic acid (C22:4n-6) was observed in the *longissimus thoracis* muscle from animals fed the Control diet, while that from animals fed the HMA diet contained the lowest (P < 0.05) proportion. Other authors have also reported that the dietary inclusion of LC n-3 PUFA is an effective means to increase their content in muscle in ruminants. In a similar study in finishing lambs, Cooper *et al.* (2004) reported an increase in the proportion of EPA and DHA in muscle when fish oil and MA were included in the diet, but the proportions of EPA and DHA were considerably higher than those obtained in the current study, reflecting the different dietary inclusion level in both studies. The increase in muscle content of EPA in the current study was achieved despite negligible amounts of this FA being present in the MA. This finding is similar to that reported by others (Phelps *et al.,*

2016a, Díaz *et al.,* 2017) and may be explained by the retro-conversion of DHA to EPA as suggested by Cooper *et al.* (2004). Alternatively, the activity of the FADS1 gene which encodes the ∆5 desaturase enzyme responsible for the conversion of 20:4n-3 to 20:5n-3 has been shown to be upregulated in liver and muscle following supplementation with MA in lambs, although the response in muscle is less predictable (Alvarenga *et al.,* 2016).

Phelps *et al.* (2016a) fed 50, 100 or 150 g per day of MA to finishing heifers and reported a quadratic response in muscle EPA and DHA concentration, reaching a maximum of 19 mg/100 g tissue. In the current study the two dietary treatments of MA equated to feeding approximately 150 and 300g MA/animal/d, with muscle DHA concentration increasing with MA inclusion level to 33 mg/100g tissue in animals fed HMA. Differences between the current study and Phelps *et al.* (2016a) may partly be explained by the basal diet, with high levels of barley being fed in the current study. High levels of starch rich ingredients such as barley is associated with a lower ruminal pH and a reduction in the biohydrogenation of PUFA in the rumen (Sinclair *et al.,* 2007). This may have increased the duodenal flow and subsequent muscle supply of EPA and DHA. Despite the absence of LC n-3 PUFA in the Control diet, the meat from cattle fed this diet was found to contain small amounts of EPA and DHA. These FA would have been synthesised by the animal via elongation and desaturation of its precursor C18:3n-3, but as discussed by Sinclair (2007), this process is relatively inefficient in ruminants compared to the dietary supply and muscle uptake of pre-formed LC n-3 PUFA.

The decrease in the proportion of C20:3n-6 and C22:4n-6 in the muscle from cattle fed either of the MA diets in the current study is consistent with previous studies, which also reported a decline in the proportion of n-6 PUFA when dietary sources of LC n-3 PUFA were fed to beef cattle (Dunne *et al.,* 2011; Phelps *et al.,* 2016a,b). In the current study, the FA composition of the intramuscular fat was analysed without separating polar and neutral lipids. However, the increase in the proportions of EPA and DHA in muscle from animals fed MA can most likely be attributed to changes in the

composition of the phospholipid (PL) fraction, as several studies have reported that the largest deposition of LC n-3 PUFA occurs in the PL fraction of the intramuscular fat. The content of PL in muscle is relatively constant and therefore any increase in the deposition of LC n-3 PUFA is probably due to the substitution for other similar FA, mainly the n-6 PUFA (Sinclair 2007; Wood *et al.,* 2008).

Muscle content of C18:3n-3 averaged 0.14 g/100 g FA and was not affected ($P > 0.05$) by treatment, reflecting the similarity in content of this FA across all three diets. Despite the replacement of LC n-6 PUFA in muscle with the inclusion of MA, there was no effect of diet ($P > 0.05$) on the proportion of C18:2n-6 or arachidonic acid (20:4n-6). The highest ($P < 0.05$) proportion of C16:0 and C18:0 was observed in muscle from cattle fed HMA, and reflects the greater dietary concentration. In contrast, dietary C18:1n-9 concentration was similar between the three treatments but its content was lower ($P < 0.05$) in muscle from cattle fed either of the MA diets. Wachira *et al.* (2002) reported that muscle concentrations of C18:1n-9 can be reduced due to the substitution by other unsaturated FA in the PL fraction of the intramuscular fat. Similarly Cooper *et al.* (2004) found that C18:1n−9 was replaced by EPA and DHA in the muscle lipid of lambs when fed LC n-3 PUFA, although Phelps *et al.* (2016a) found no effect of the inclusion of MA in the diet on muscle C18:1n-9.

Intramuscular fat indices and human health:

In the European Union the reference intake values of EPA plus DHA for humans is 250 mg/day (EFSA, 2010). A 100 g serving of beef meat from the animals receiving the LMA treatment would supply approximately 62.2 mg of these LC n-3 PUFA, while a 100 g serving of meat from the HMA fed animals would supply approximately 71.2 mg. These values represent a supply of approximately 25 to 28% of the recommended daily requirement. A food product can be marketed within Europe as being a “source of omega-3 fatty acids” when it contains at least 40 mg/100g of EPA plus DHA, and as

being “high in omega-3 fatty acids” when it contains at least 80 mg/100g (Commission Regulation of the European Union, 2010). Meat from animals fed the Control treatment contained 25.4 mg of EPA plus DHA/100g, and therefore does not meet the minimum content. In contrast, meat from cattle fed either of the diets containing MA meet the required levels of EPA plus DHA to be labelled as a food product that is a “source of omega-3 fatty acids”, but not to be “high in omega-3” fatty acids.

There was no effect ($P > 0.05$) of dietary treatment on the content of SFA, MUFA or PUFA in the *longissimus thoracis* muscle (Table 2). Similarly, the dietary inclusion of MA did not alter ($P > 0.05$) the polyunsaturated to saturated (P:S) ratio of muscle, with a value of approximately 0.2 for all treatments. This ratio is similar to that reported by Hopkins *et al.* (2014) and Phelps *et al.* (2016a), but lower than that of Cooper *et al.* (2004), who reported a P:S ratio of 0.46 in muscle from lambs when fed rumen protected linseed and MA. However, the dietary addition of MA in the current study did lead to a large improvement ($P < 0.05$) in the n-6:n-3 ratio, being 2.9, 1.9 and 1.6 in the muscle from animals fed the Control, LMA or HMA diet respectively. A recommended value for the n-6:n-3 ratio in the human diet is approximately 2, and therefore the meat from cattle fed either diet containing MA comply with this, while those fed the Control diet in the current study, or cattle fed similar concentrate based diets (Dunne *et al.,* 2011; Phelps *et al.,* 2016a), do not. This improvement in the n-6:n-3 ratio is consistent with other studies where sources of LC n-3 PUFA have been included in the diet of ruminants (e.g. Wachira *et al.,* 2002; Phelps *et al.,* 2016a).

Fatty acid content of the subcutaneous adipose tissue

The FA content of the subcutaneous adipose tissue was analysed in the current study as some consumers, when given the choice, may choose to consume the fat on a portion of meat, whilst others may prefer to cook in tallow rather than vegetable oil. The total FA content in the subcutaneous adipose tissue was similar in animals receiving any

of the three diets, with an average value of 79753 mg/100 g of tissue ($P > 0.05$; Table 3). The subcutaneous adipose tissue from heifers fed the Control diet had the highest ($P < 0.05$) content of C18:2n-6, C20:3n-6 and total n-6 FA, while those fed the HMA diet had the lowest. In contrast, the content of C22:5n-3 was highest ($P < 0.05$) in the subcutaneous adipose tissue from animals fed the LMA or HMA diet, and lowest in those fed the Control diet. The highest ($P < 0.05$) content of C14:0, C16:0 and C18:1 *trans*-9 was observed in the subcutaneous adipose tissue from cattle fed the HMA diet, whereas the levels of C18:1 *trans*-11, C18:1n-9 and C18:2 *cis*-9, *trans*-11 were highest ($P < 0.05$) in animals fed the Control diet. The minor effect of the inclusion of MA in the diet on the proportion of EPA and DHA in the subcutaneous adipose tissue in the current study reflects the low incorporation of these FA in the triacylglycerol fraction and the low proportion of PL found in the adipose tissue (Cooper *et al.,* 2004). Contrary to this, Meale *et al.* (2014) reported a large increase of LC n-3 PUFA in subcutaneous adipose tissue and perirenal adipose tissue of lambs when MA was fed.

Sensory quality and shear force

Steaks from cattle fed HMA were scored as having the highest ($P < 0.05$) intensity of the attribute “seaweedy flavour”, followed by the steaks from cattle fed the LMA diet and the Control animals the lowest (Table 4 and Figure 1). The attribute “seaweedy flavour” was defined by the sensory panel as “the mixture of grassy, seaweedy and/or fishy flavours”. This finding is consistent, although less pronounced than that reported by Vatansever *et al.* (2000), where steaks from cattle fed fish oil were described as containing a higher “fishy flavour” than those from animals fed a control diet. Similarly, Phelps *et al.* (2016a,b) reported an increase in off-flavour intensity in beef from animals fed higher levels of MA, whilst Nute *et al.* (2007) reported that meat from lambs fed dietary sources of LC n-3 PUFA had higher scores for a number of attributes including “fishy flavour”, “rancid flavour” and “abnormal lamb flavour”. Vatansever *et al.* (2000) also reported a decrease

in “beef flavour” and increase in “livery flavour” in steaks from cattle fed fish oil, although no differences were detected in the current study. There was also no effect ($P > 0.05$) of dietary treatment in the current study on any of the other sensory attributes, except for “tenderness”, with meat from animals fed the HMA diet having the highest score (i.e. most tender), while those from animals fed LMA had the lowest score ($P < 0.001$). Similar to the sensory evaluation, steaks from animals fed LMA had the highest ($P < 0.05$) shear force and toughness, while those from cattle fed the HMA diet had the lowest. Other authors have found little effect of LC n-3 PUFA content on toughness (Nute *et al.,* 2007; Phelps *et al.,* 2016a) and reasons for the differences in the current study are unclear.

There was a positive correlation ($P < 0.05$) between the proportion of EPA and DHA in the intramuscular fat and “seaweedy flavour” scores in steaks, with DHA having the strongest positive correlation coefficient of 0.6 followed by EPA at 0.48 (Supplementary Table S2). This is the first study to report this finding in cattle fed MA, but is consistent with the results of Nute *et al.* (2007) who reported a positive correlation between the “fishy flavour” in lamb meat and the proportion of EPA and DHA in the intramuscular fat. The volatile compounds formed in cooked meat are mainly derived from thermal degradation of lipids and the Maillard reaction (Calkins & Hodgen, 2007). Thermal oxidation of lipids produces volatile compounds such as aliphatic aldehydes, ketones, and alcohols, which contribute to the flavour of meat (Mottram, 1994). Elmore *et al.* (1999), reported that cooked beef meat with high amounts of LC n-3 PUFA produced higher concentrations of lipid oxidation products, an effect that was also demonstrated in cooked lamb meat (Elmore *et al.,* 2005). Elmore *et al.* (1999) also discussed that high contents of LC n-3 PUFA in meat could catalyse the degradation of other FA. Therefore, due to the low oxidative stability of LC n-3 PUFA, modifying its concentration in meat would result in alterations to the composition of the aroma volatiles produced during cooking, with lipid oxidation being related to the generation of off-odours and off-flavours such as “fishy”, “greasy”, “rancid” and “abnormal” (Wood *et al.,* 2008). However, none of these attributes were detected in the current study.

Few studies have documented the impact of MA on carcase quality or performance of beef animals. The lack of an effect of treatment in the current study (Table 5) is in agreement with studies that have fed MA to lambs (Cooper *et al.,* 2004; Hopkins *et al.,* 2014) or fish oil to cattle (Scollan *et al.,* 2001). In contrast, Díaz et al. (2017) reported a decrease in daily weight gain in growing lambs when MA was included at 2% of the diet. Additionally, Stokes *et al.* (2015) reported a linear decrease in whole tract dry matter digestibility when partially deoiled MA was fed to lambs. Demirel *et al.* (2004) also reported an increase in carcase fat score when fish oil plus linseed was fed to sheep, which was attributed to an increase in the total FA content of the neutral lipid fraction of the muscle fat. Feeding levels of MA above those used in the current study should therefore take into account the potential decrease in diet digestibility and increase in carcase fat content.

Conclusions

The present study demonstrated that feeding microalgae is an effective means to increase the content of LC n-3 PUFA in beef meat, particularly the nutritionally beneficial EPA and DHA. The increase in EPA and DHA in meat was proportional to the inclusion rate of microalgae in the diet, and could permit the meat to be labelled as a source of omega-3 fatty acids, providing consumers with the choice of a meat containing healthier characteristics. However, the inclusion of microalgae was associated with the development of some undesirable flavours in meat. Future research should focus on evaluating different inclusion rates of microalgae in the diet in combination with additional antioxidants on lipid oxidation and sensory attributes.

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Table 1 Fatty acid composition (% of total fatty acids), and total fatty acid content (mg/100 g of fresh tissue) of the *longissimus thoracis* muscle of beef heifers fed a concentrate based diet that contained no microalgae (Control), a low inclusion of microalgae (LMA) or a high inclusion of microalgae (HMA)

Fatty acid	**Control**	**LMA**	**HMA**	**s.e.d.**	***P*-value**
C14:0	2.12	2.15	2.30	0.155	0.497
C16:0	22.97^{b}	24.24^{ab}	25.05^{a}	0.630	0.013
C16:1n-7	3.35^{a}	3.24^{ab}	2.96^{b}	0.155	0.053
C18:0	12.25^{ab}	11.61^{b}	12.54^{a}	0.322	0.029
C18:1*trans*-11	1.99^{a}	1.91^{ab}	1.79^{b}	0.069	0.031
C18:1n-9	36.45^{a}	35.40^{a}	33.24^{b}	0.677	<0.001
C18:1*trans*-9	0.45^{b}	0.91^{a}	1.15^{a}	0.151	<0.001
C18:2n-6	2.99	2.66	2.60	0.273	0.319
C18:2*cis*-9, *trans*-11	0.30	0.24	0.23	0.031	0.081
C18:2*trans*-10,*cis*-12	0.03	0.03	0.03	0.003	0.669
C18:3n-3	0.15	0.14	0.12	0.010	0.083
C20:3n-6	0.27^{a}	0.19^{b}	0.20^{b}	0.027	0.014
C20:4n-6	1.16	0.95	1.04	0.095	0.103
C20:5n-3	0.50^{c}	0.92^{b}	1.20^{a}	0.080	<0.001
C22:4n-6	1.25^{a}	1.17^{a}	1.07^{b}	0.038	0.001
C22:5n-3	0.77^{a}	0.53^{b}	0.49^{b}	0.068	0.001
C22:6n-3	0.31^{c}	0.89^{b}	1.05^{a}	0.053	<0.001
Σ n-6 fatty acids[1]	5.68	4.96	4.92	0.388	0.117
Σ n-3 fatty acids[2]	1.73^{b}	2.47^{a}	2.86^{a}	0.154	<0.001
Total FA, mg/100g fresh tissue	3359	3683	3241	302.9	0.341

[1]Σ n-6 fatty acids= C18:2n-6 + C20:3n-6 + C20:4n-6 + C22:4n-6.
[2]Σ n-3 fatty acids= C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3.
[a,b,c] Means in a row with different superscripts letters differ ($P < 0.05$).

Table 2 Fatty acid classes (mg/100 g of fresh tissue) and ratios of the *longissimus thoracis* muscle of beef heifers fed a concentrate based diet that contained no microalgae (Control), a low inclusion of microalgae (LMA) or a high inclusion of microalgae (HMA)

Item	Control	LMA	HMA	s.e.d.	*P*-value
SFA[1]	1270	1399	1295	129.2	0.580
MUFA[2]	1464	1575	1307	136.7	0.172
PUFA[3]	242	272	254	11.7	0.063
P:S ratio[4]	0.21	0.20	0.20	0.017	0.898
n-6:n-3 ratio[5]	2.88[a]	1.91[b]	1.61[c]	0.082	<0.001

[1]Saturated fatty acids.
[2]Monounsaturated fatty acids.
[3]Polyunsaturated fatty acids.
[4]P:S= total polyunsaturated to total saturated fatty acids ratio.
[5]n-6:n-3= total n-6 (C18:2n-6 + C20:3n-6 + C20:4n-6 + C22:4n-6) to n-3 fatty acids (C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3).
[a,b,c] Means in a row with different superscripts letters differ ($P < 0.05$).

Table 3 Fatty acid composition (% of total fatty acids) and fatty acid content (mg/100 g of tissue) of the subcutaneous adipose tissue of beef heifers fed a concentrate based diet that contained no microalgae (Control), a low inclusion of microalgae (LMA) or a high inclusion of microalgae (HMA)

Fatty acid	Control	LMA	HMA	s.e.d.	*P*-value
C14:0	2.93[b]	3.07[ab]	3.55[a]	0.211	0.022
C16:0	25.40[c]	27.38[b]	29.43[a]	0.612	<0.001
C16:1n-7	4.18	4.18	4.05	0.389	0.932
C18:0	11.38	11.34	11.74	0.677	0.816
C18:1 *trans*-11	1.82[a]	1.73[a]	1.56[b]	0.067	0.003
C18:1n-9	38.20[a]	35.46[b]	31.81[c]	0.847	<0.001
C18:1 *trans*-9	0.70[c]	1.78[b]	3.10[a]	0.398	<0.001
C18:2n-6	1.04[a]	0.92[ab]	0.85[b]	0.066	0.026
C18:2 *cis*-9, *trans*-11	0.24[a]	0.19[ab]	0.16[b]	0.024	0.013
C18:2 *trans*-10,*cis*-12	0.02[b]	0.04[a]	0.05[a]	0.008	0.005
C18:3n-3	0.11	0.09	0.09	0.007	0.057
C20:3n-6	0.04[a]	0.03[b]	0.03[b]	0.005	0.017
C20:4n-6	0.04	0.03	0.04	0.005	0.077
C20:5n-3	0.09	0.06	0.07	0.013	0.148
C22:4n-6	1.27	1.23	1.22	0.041	0.326
C22:5n-3	0.04[b]	0.06[a]	0.06[a]	0.007	0.023
C22:6n-3	0.14	0.10	0.13	0.021	0.350
Σ n-6 fatty acids[1]	2.40[a]	2.21[ab]	2.14[b]	0.075	0.008
Σ n-3 fatty acids[2]	0.37	0.32	0.34	0.034	0.323
Total fatty acids (mg/100 g of tissue)	82221	79438	77601	2121.8	0.119

[1]Σ n-6 fatty acids= C18:2n-6 + C20:3n-6 + C20:4n-6 + C22:4n-6,
[2]Σ n-3 fatty acids= C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3.
[a,b,c] Means in a row with different superscripts letters differ, ($P < 0.05$).

Table 4 Sensory attributes shear force and toughness of cooked steaks from beef heifers fed a concentrate based diet that contained no microalgae (Control), a low inclusion of microalgae (LMA) or a high inclusion of microalgae (HMA)

Attribute	Control	LMA	HMA	s.e.d.	*P*-value
Odours[1]					
Dairy	2.9	2.6	2.9	0.34	0.664
Beefy	6.3	5.9	5.9	0.33	0.309
Roast	4.8	4.4	4.5	0.37	0.519
Flavours[1]					
Beefy	7.4	7.1	6.9	0.29	0.258
Roast	5.7	5.1	5.2	0.38	0.216
Sweet	2.6	2.4	2.4	0.28	0.812
Salty	1.8	1.9	1.9	0.23	0.877
Livery	2.4	2.6	2.8	0.22	0.214
Seaweedy	0.7^{c}	1.3^{b}	1.8^{a}	0.21	<0.001
Overall richness of flavour	7.2	6.9	7.1	0.27	0.539
Aftertastes[1]					
Metallic	3.4	3.4	3.3	0.34	0.972
Umami	5.4	5.2	5.0	0.39	0.475
Fat coating	2.7	2.9	2.7	0.29	0.694
Livery	2.4	2.5	2.6	0.24	0.728
Unusual	0.9	1.1	1.3	0.23	0.138
Texture					
Tenderness[2]	6.6^{ab}	6.0^{b}	7.2^{a}	0.31	<0.001
Juiciness[3]	6.5	6.0	6.3	0.33	0.363
Fat mouth feel[1]	2.5	2.4	2.4	0.28	0.879
Shear force (N)	40.7^{b}	46.3^{a}	35.2^{c}	2.13	<0.001
Toughness (N.s)	129.9^{ab}	140.5^{a}	111.1^{b}	8.52	0.010

[1] scale 1-15; 0 = weak, 15 = strong

[2] scale 1-15; 0 = hard to chew, 15 = melt in the mouth

[3]scale 1-15; 0 = dry, 15 = juicy

[a,b,c] Means in a row with different superscripts letters differ, ($P < 0.05$).

Table 5 Carcase characteristics and performance of beef heifers fed a concentrate based diet that contained no microalgae (Control), low inclusion of microalgae (LMA) or a high inclusion of microalgae (HMA)

Item	Control	LMA	HMA	s.e.d.	Significance
Initial live weight (kg)	509	509	509	6.87	1.000
Slaughter weight (kg)	659	661	660	10.16	0.970
DLWG[1] (kg/d)	1.57	1.59	1.58	0.087	0.968
Carcase weight (kg)	349	351	351	8.48	0.968
Carcase conformation[2]	8.0	8.4	8.0	0.41	0.537
Carcase fat class[3]	11.3	11.7	11.1	0.62	0.625

[1]Daily live weight gain.
[2]Conformation, 1= poor to 15 = excellent.
[3]Fat Class, 1 = lean to 15 = fat.

Table S1 Fatty acid and chemical composition of diets fed to beef heifers that contained no microalgae (Control), a low inclusion of microalgae (LMA) or a high inclusion of microalgae (HMA)

	Control	LMA	HMA
Fatty acid composition (g/kg DM)			
C14:0	0.17	0.62	1.01
C16:0	2.89	7.66	12.08
C18:0	0.23	0.33	0.43
C18:1n-9	1.70	1.69	1.65
C18:2n-6	7.33	7.14	7.05
C18:3n-3	0.84	0.84	0.81
C20:5n-3	ND[2]	0.03	0.05
C22:5n-3	ND[2]	0.48	0.95
C22:6n-3	ND[2]	2.04	4.08
Total FA content	13.3	21.2	29.2
Chemical composition (g/kg DM)			
Dry matter (g/kg)	873	867	870
Organic matter	965	959	953
Crude protein (N x 6.25)	139	126	126
Neutral detergent fibre	259	241	244

[1]Not detected

Table S2 Correlation coefficients (*P*-value) between "seaweedy flavour" in steaks and the proportion of n-3 PUFA in the muscle from beef heifers fed diets varying in their inclusion of microalgae

	C18:3n-3 α-linolenic	**C20:5n-3 EPA[1]**	**C22:5n-3 DPA[2]**	**C22:6n-3 DHA[3]**
Seaweedy flavour	-0.43 (0.019)	0.48 (0.007)	-0.38 (0.037)	0.60 (0.000)

[1]Eicosapentaenoic acid. [2]Docosapentaenoic acid. [3]Docosahexaenoic acid.