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Fatty acid profile and intramuscular fat concentration of *Musculus longissimus thoracis* in bulls fed grass silage harvested at one of three maturity stages, either with or without concentrate supplementation

Åshild T. Randby, Laila Aass and Anna Haug

Faculty of Biosciences, Norwegian University of Life Sciences, Ås, Norway

ABSTRACT

The study investigated the effect of plant maturity of grass silage on intramuscular fat (IMF) concentration and fatty acid profile in *M. longissimus thoracis* of bulls. From 7 to 8 months of age until slaughter, 36 bulls of Norwegian Red were offered grass silage harvested at three maturity stages *ad libitum*, with or without concentrate supplement. Increasing plant maturity decreased the proportion of α-linolenic acid in IMF ($P = 0.04$). Concentrate supplementation increased the proportions in IMF of linoleic acid ($P < 0.001$) and C20:3n-6 ($P < 0.008$), decreased all analysed n-3 polyunsaturated fatty acids ($P \leq 0.02$), conjugated linoleic acid ($P < 0.01$) and trans vaccenic acid ($P < 0.001$). Polyunsaturated fatty acids n-6/n-3 ratios were in the range 1.0–2.1 and increased with plant maturity and concentrate supplementation. Results suggest that 'grass-fed beef' also may be produced indoors with grass silages in regions with short grazing season.

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Grass-fed beef; carcass; EPA; DHA; n-6/n-3 ratio

Introduction

Consumers increasingly demand healthy food, and questions regarding nutritional quality in grass-fed and grain-fed cattle are raised (Daley et al., 2010). Increased intake by consumers of n-3 polyunsaturated fatty acids (PUFA), and particularly of the long-chain PUFA (chain length ≥ 20 C) eicosapentaenoic acid (EPA; C20:5 n–3) and docosahexaenoic acid (DHA; C22:6 n–3), is beneficial to human health (World Health Organisation, 2003). Several theories link the consumption of long-chain n-3 PUFA to reducing the incidence of disease conditions including cardiovascular disease, inflammatory diseases such as arthritis, and mental health disorders (Clayton, 2014). Also, the long-chain PUFA docosapentaenoic acid (DPA; C22:5 n-3) that is found in higher concentrations in beef than C20:5 n-3 and C22:6 n-3, has gained attention due to several possible health benefits in humans (Clayton, 2014). Plants are the primary source of n-3 PUFA due to their unique ability to synthesise *de novo* α-linolenic acid (C18:3 n-3), which is the building block of the n-3 series of essential fatty acids (Scollan et al., 2006). Elongation and desaturation of this fatty acid result in the synthesis of C20:5 n-3, C22:5 n-3 and C22:6 n-3 (Scollan et al., 2006; Kaur et al., 2011). Although a portion of dietary unsaturated fatty acids is biohydrogenated in the rumen, an increasing level in the forage results in increasing concentrations in body fat (Scollan et al., 2014). Reducing the extent of ruminal biohydrogenation may also contribute to alter the fatty acid composition of intramuscular fat (IMF; Scollan et al., 2006). Intramuscular fat in beef from cattle finished on pasture or on a high proportion of forage relative to grain-based concentrates contains a lower n-6/n-3 ratio of fatty acids (Scollan et al., 2006) and a higher proportion of conjugated linoleic acid (CLA; C18:2cis9, trans11) than in beef from concentrate-finished cattle (Nuernberg et al., 2005). Because long-chain PUFA easily oxidises, increasing proportions of PUFA in beef may reduce shelf life, but vitamin E, which is an antioxidant normally abundant in fresh grasses, may help to stabilise the products (Scollan et al., 2006).

The higher proportion of the beneficial n-3 PUFA often found in fat in muscles from grass-fed, compared to grain-fed cattle (Daley et al., 2010) may be caused by several feed factors such as forage species, wilting, silage fermentation, and the amount and composition of concentrate. Wilting of forages represents oxidative losses of PUFA (Boufaied et al., 2003; Dewhurst et al., 2006; Van Ranst et al., 2009). Halmemies-Beauchet-Filleau et al. (2013) found that drying of grass resulted in substantial

CONTACT Åshild T. Randby ashild.randby@nmbu.no

decreases in fatty acid content in g/kg dry matter (DM), particularly of 18:2 n-6 and 18:3 n-3, whereas ensiling resulted in minimal losses. However, compared with fresh grass at 180 g DM/kg, and grass silage wilted to 240 g DM/kg, feeding diets based on grass hay lowered the extent of lipolysis and biohydrogenation of unsaturated fatty acids in the rumen. In cows, the omasal flow of C18:2 n-6 and C18:3 n-3 were similar when fresh grass or the corresponding slightly wilted silage was fed (Halmemies-Beauchet-Filleau et al., 2013). Fatty acid concentrations can vary between different forage species and cultivars, for example, the concentration of C18:2 n-6 was higher, and the concentration of C18:3 n-3 was lower in legumes compared with grasses (Boufaied et al., 2003). In the same study, a large variation was observed among species within each family of grasses and legumes. Berthiaume et al. (2015) found improved fatty acid composition traits in muscle from beef calves fed red clover (*Trifolium pratense*) – timothy (*Phleum pratense*) versus tall fescue (*Festuca arundinacea*) silages. Immature grasses contain more fat than mature grasses, and the maturity stage may influence the fatty acid composition of plant lipids (Dewhurst et al., 2001). Correspondingly in timothy, Boufaied et al. (2003) observed that DM concentrations of total fatty acids and all the individual major plant fatty acids palmitic acid (C16:0), C18:2 n-6 and C18:3 n-3 significantly decreased between stem elongation and early flowering.

Data for the present study were from a previous study where growth performance, carcass measures and feed efficiency of bulls were presented by Randby et al. (2010), and where Bonesmo and Randby (2011) studied how varying prices on silage and concentrate influenced finishing profitability of dairy bulls, and where Åby et al. (2019) evaluated greenhouse gas emissions from the same beef production systems using a farm-scale model. In the future, the healthiness of food products from meat animals may impact the demand and price of varying products, i.e. for 'grass-fed beef' compared with grain-fed beef (Scollan et al., 2006). Because the grazing season is indeed short in cold regions, it was of interest to study if 'grass-fed beef' also could be produced indoors on grass silage. The main objective of the present study was to investigate if plant maturity in timothy-dominated grass silage, and concentrate supplementation, influenced fatty acid profile, IMF, and α-tocopherol concentrations in beef from bulls slaughtered at 575 kg target live weight (LW). We hypothesised that beef produced from silage of the least mature grass crop would obtain the lowest n-6/n-3 ratio in the fatty acid profile of IMF, and that concentrate supplementation would increase the n-6/n-3 ratio.

Materials and methods

Experimental design and animal management

The study was undertaken at the Norwegian University of Life Sciences, Ås, Norway (59°40′N, 10°47′E) in 2006–2007. A timothy, meadow fescue (*Festuca pratensis*) and red clover sward that on average consisted of 95% grasses and 5% red clover was cut at each of three maturity stages (harvesting times) in the primary growth: 30 May to 1 June (H1), 6–8 June (H2) and 14–16 June (H3). The maturity of timothy, the dominating grass species at harvest, given as mean stage by weight (MSW; Moore et al., 1991) was 2.44, 2.73 and 3.30 for the three harvesting times, respectively. This indicated that H1 and H2 were dominated by tillers in stem elongation with 2 and 3 visible nodes, respectively, and H3 was dominated by tillers with visible heads, but without head stems (early heading). The crop was wilted for 2–7 h during daytime or 14–22 h over night with no precipitation, applied a formic acid-based silage additive and preserved in round bales.

The study included 36 Norwegian Red bulls from the University herd. When calves were on average 7 months old they were moved to the experimental tie-up stall, and on average 7.8 months old they were allocated to six blocks based on live weight (LW), age, daily live weight gain (LWG) since birth and intake of silage in a 14-d preliminary period. One bull from each block was allocated to each of the six treatment groups. The treatment groups were randomly allocated to the experimental feeding regime.

The six dietary treatments included silage from H1, H2 and H3 offered *ad libitum* as the sole feed or supplemented with concentrate. From the start of the experiment (average LW 288 kg), the supplemented bulls received 2 kg concentrate daily, increasing to 3 kg at 385 kg LW and to 4 kg at 500 kg LW, individually for each animal. All bulls were fed 100 g/d of a mineral and vitamin mix (Pluss Multitilskudd Appetitt, Felleskjøpet Fôrutvikling, Trondheim, Norway) containing (g/kg) 110 Ca, 70 P, 65 Mg, 90 Na, 0.4 Cu, 0.013 Se, 0.013 Co, 3.33 Zn, 2.65 Mn, 0.1 I, and per g: 400 IU Vitamin A, 120 IU Vitamin D3, 2 mg α-tocopheryl-acetate.

Silage and concentrate were fed separately twice daily and individual intakes recorded. Bulls were weighed three consecutive days initially and prior to slaughter at 575 kg target LW, and two consecutive days every four weeks throughout. Further details on silage harvesting, including the type of round baler, chop length of crop, type and application rate of silage additive, type of wrapping machine, plastic quality and the number of layers are given by Randby et al. (2010) as well as further details on experimental management.

Feed composition and feed analyses

The ingredient composition of the concentrate mixture was: 0.3 oats, 0.18 peas, 0.179 barley, 0.1 wheat, 0.1 wheat bran, 0.06 extracted, heat-treated rape seed meal, 0.045 molasses, 0.036 minerals and vitamins, including 0.01 of a micro mineral and vitamin mix. The concentrate was supplied 5000 IU Vitamin A, 2000 IU Vitamin D3 and 45 mg α-tocopheryl-acetate per kg. Samples of concentrates were taken every 14 d. A core sample was taken from each silage bale daily during feeding, and frozen on – 18°C.

For fatty acid composition, samples of each of the three silages, and the concentrate, bulked over the entire experimental period, were freeze-dried and milled through a 0.5 mm screen (Retsch hammer mill, Haan, Germany) before being analysed in duplicate. The milled feed samples were directly methylated according to O'Fallon et al. (2007) and analysed with a Thermo Finnigan Focus GC with a split/splitless Focus GC+ injector, and flame ionisation detection (Thermo-Finnigan, Milan, Italy). The separation was performed with a Restek RT-2560 (100 m × 0.25 mm internal diameter × 0.2 µm film thickness) column (Restek U.S., 110 Benner Circle, Bellefonte, PA, USA). Temperature programme, initial: 70°C with 2 min hold, then an increase to 160°C with 20°C/min, followed by a 40 min hold. Then an increase to 230°C with 2°C/min with a subsequent 10 min hold. Carrier gas was He with a pressure of 270 kPa. The fatty acid analysis was performed by auto-injection of 2 µL of each sample at a split ratio of 1:30, constant flow mode, average velocity 16.8 cm/s. The flame ionisation detector temperature was 230 °C. The run time for a single sample was 91.5 min.

Crude fat analyses in silage and concentrates were done after hydrolysis of the samples with 3 *M* HCl, extraction with petroleum ether before distillation of the eluent followed by drying and weighing of the residues. Feed sample preparation, milling procedures and all feed analyses, including digestibility trials with sheep, were done as described by Randby et al. (2010). Metabolisable energy was calculated from feed chemical composition and sheep digestibility (Van Es, 1978).

Transport of animals, slaughter protocol and sampling

When target LW 575 kg was obtained, one to five bulls were slaughtered at each of 19 slaughter dates. Slaughter was undertaken at a commercial abattoir located in Tønsberg, 52 km from Ås. The transport was done by a professional transporter, and included 1 h on the road plus a 30 min ferry trip, and up to 30 min to wait on the quay. All bulls left in the morning and were slaughtered 3–5 h after removal from the stable. Carcasses were subjected to electrical stimulating using M 300-HS (Norsystem AS, Asker, Norway) low voltage unit delivering 90 v (pulse width 5 ms, pulse interval 70 ms (14.3 Hz)), for 32 s. Carcass conformation and fat classification were determined by visual assessment by an accredited classifier according to the European Carcass Classification Scheme (EU Beef Carcass Classification Scheme, 2020). Trained cutters removed the *M. longissimus thoracis* (LT) from the site between the 11th and 13th thoracic vertebrae (in total 6–8 cm) from one side of the carcass 45–60 min post mortem. The 2 cm back part of this, divided perpendicularly to the fibre direction, was vacuum packed and later used for chemical analyses. The slices were conditioned at 11°C for the first 24 h, thereafter aged at 4°C the following 13 days, and further stored at −20°C for on average 20 months until analyses. On average 6 days after the slaughter, cold carcass weight was recorded, and one carcass side of each animal was dissected into 9 primal cuts, lean trim, fat trim, bone and waste (glands, veins, residues of tallow, etc.) for determination of proportions of fat, lean, bone and waste using a commercial cutting procedure according to Krog et al. (1997). Daily carcass gain was estimated assuming a dressing proportion of 0.50 at grouping (Berg & Matre, 2001).

Meat analyses

The slices of LT were thawed, cut clear of visible fat and sinews, and 50 g was homogenised with water 1:1. For determination of α-tocopherol, 1.0 g of homogenate was mixed with 3 mL 2-propanol containing 2.5 µg/ml 2-propanol of the internal standard tocol and 20 µg/ml 2-propanol of butylated hydroxytoluene as an antioxidant. After mixing for 15 min and centrifugation (10 min, 4000 g at 10°C), an aliquot of 20 µL was injected from the supernatant into the HPLC system. HPLC was performed with an HP 1100 liquid chromatograph (Agilent Technologies, Palo Alta, CA, USA) with a HP1100 fluorescence detector, em: 295 nm ex: 330 nm. α-tocopherol was separated on a 4.6 mm × 50 mm reversed-phase column. A calibration curve was made from analysis of an ethanol solution enriched with a known concentration of α-tocopherol.

For determination of LT fatty acid composition, 0.5 g of meat homogenate was directly methylated according to O'Fallon et al. (2007) and analysed with a 6890N GC with a split/splitless injector, a 7683B automatic liquid sampler, and flame ionisation detection (Agilent Technologies, Palo Alto, CA). The separation was performed with a DB-23 (60 m × 0.25 mm internal diameter ×

0.25 µm film thickness) column (Agilent Technologies, Palo Alto, CA). Temperature programme, initial: 120°C with 1 min hold, ramp 7°C/min to 230°C with 12 min hold. The carrier gas was H_2 with a pressure of 95.1 kPa. Fatty acid analysis was performed by auto-injection of 1 µL of each sample at a split ratio of 1:10, constant flow mode, average velocity of 28 cm/s. The flame ionisation detector temperature was 260°C with H_2, air and N_2 make-up gas flow rates of 40, 450 and 45 ml/min respectively. The sampling frequency was 10 Hz. The run time for a single sample was 28.71 min. Fatty acids were identified by comparing retention times with reference standard 37-component FAME-mix from Supelco Analytical (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany).

For determination of IMF in LT, lipids were extracted from 4 g of meat homogenate using chloroform:methanol (2:1, v/v) as described by Folch et al. (1957). The amount of IMF was determined by weighing the extract after evaporation at 40°C.

For comparisons with recommended nutritional levels for humans, the given fatty acid proportions of some quantitatively important fatty acids and all n-6 and n-3 acids (Table 4) were converted to mg/100 g muscle by using the IMF concentration (Table 3), and the proportion of fatty acids in fat from lean beef meat (≈ 0.916; Food composition data, Appendix 5, FAO, https://www.fao.org/3/y4705e/y4705e22.htm downloaded in September 2020).

Statistical analyses

Data were analyzed using the PROC MIXED procedure of SAS (release 9.4, 2002–2012; SAS Institute Inc., Cary, NC, USA), by a model including the 6 blocks, the 3 harvest times for grass silage (H), 2 concentrate levels (C: without concentrates, or concentrate supplemented) and the H × C interaction. Results were presented as least square (LS) means. Treatment means were separated using the PDIFF statement. For the intake of silage DM, fat, and ME, the initial LW of bulls was found significant and used as a covariate. Cold carcass weight was tested as a covariate for carcass assessments and found significant, and used, for carcass conformation, only. Slaughter date as a fixed RANDOM effect improved Bayesian Information Criteria (BIC) for all carcass assessments, and was therefore included. Initial LW and cold carcass weight of bulls were tested as a covariate for IMF but found not to be significant. Intramuscular fat was tested as a covariate for α-tocopherol measures and fatty acid profile of LT. It was not significant for α-tocopherol measures, but found significant and included in the analyses of 16 of the 24 fatty acids given in Table 4, including all fatty acids with more than one double bond. Intramuscular fat as a covariate was also found to be significant and used for the other measures of the fatty acid profile in Table 4, apart from the n-6/n-3-ratios for C18 acids and all PUFA. Fatty acid concentrations in mg/100 g muscle (Table 5) were analysed without the use of covariates. Simple correlations were estimated with the PROC REG procedure, without the use of covariates.

Results

Feed composition

Grass silages from all three harvesting times were well fermented (Table 1), with low concentrations of lactic and acetic acid, only traces of butyric acid, and high concentrations of water-soluble carbohydrates. The concentrations in DM of crude protein, fat, digestible organic matter (DOM) and metabolisable energy (ME) decreased with increasing plant maturity, whereas NDF concentration increased. Concentrate contained similar levels of protein, fat, DOM and ME as H1 silage, but less of NDF. The proportion of the major fatty acid in silages, C18:3 n-3, decreased with increasing plant maturity (Table 2). A weaker but similar trend was found for C18:2 n-6, whereas the proportion in silage fat of all other analysed fatty acids increased with increasing plant maturity (Table 2). Because fat concentration in silage decreased with increasing maturity (Table 1), all quantitatively important fatty acids in feeds (those that constituted more than 1 g/100 g fatty acid in the profile) were present in silage DM in the highest concentrations in the earliest harvested silage (Table 2).

Whereas C18:3 n-3 was by far the most abundant fatty acid in grass silage (46 g/100 g fatty acid, on average over silages), it constituted less than 1 g/100 g fatty acid in concentrates (Table 2). Correspondingly, oleic acid, C18:1c9, was the most abundant acid in concentrates (35 g/100 g fatty acid) but constituted only about 3 g/100 g fatty acids in silage. C18:3 n-3 and C16:0 were major fatty acids in both silage and concentrates, with roughly 15 g/100 g fatty acid of each in silage and 24 g/100 g fatty acid of each in concentrates. Whereas the n-6/n-3 ratio was only 0.33 in H1 and H2, and 0.36 in H3, it was nearly 100-fold higher in concentrates (29.2).

Feed intake and weight gain

Silage intake was high when H1 or H2 silage was offered as the sole feed, and decreased with increasing plant maturity at harvest and with concentrate

Table 1. Chemical composition of silages and concentrate.

	Harvesting time for silage [f]							
	H1		H2		H3		Concentrate	
	mean	s.d.[e]	mean	s.d.	mean	s.d.	mean	s.d.
Dry matter (DM), g/kg	299	39.6	271	29.1	322	44.0	923	12.1
Crude protein, g/kg DM	166	13.2	145	9.2	113	5.5	165	5.2
Crude fat, g/kg DM	38.0	3.71	29.6	1.82	25.2	1.59	44.5	3.9
NDF [a], g/kg DM	477	16.1	533	19.1	601	18.2	207	4.1
Starch, g/kg DM							420	19.2
WSC [b] g/kg DM	82.3	15.9	80.6	23.6	63.0	12.1	36.8	
Lactic acid, g/kg DM	62.4	18.4	74.7	14.5	41.1	9.0		
Acetic acid, g/kg DM	6.3	2.00	7.2	2.31	5.2	2.12		
DOMD [c]	0.747	0.017	0.708	0.017	0.647	0.022	0.725	0.024
ME [d], MJ/kg DM	11.4	0.26	10.7	0.27	9.6	0.33	11.6	0.32

For silages: DM, WSC, lactic acid, acetic acid: n = 12 for harvesting time 1, and 13 for harvesting times 2 and 3. Crude protein and NDF: n = 5. Silages and concentrates: DOMD and ME: n = 3. For concentrates: n = 6 where s.d. is given, otherwise n = 1.
Only traces of butyric acid were detected.
[a]Neutral detergent fibre analysed using α-amylase and reported free from residual ash.
[b]Water-soluble carbohydrates.
[c]Digestible organic matter in DM.
[d]Metabolisable energy
[e]Standard deviation.
[f]Maturity stage of timothy, the dominating grass species at harvest, given as mean stage by weight (MSW) was 2.44, 2.73 and 3.30 for H1, H2 and H3, respectively (Moore et al., 1991).

supplementation ($P < 0.001$; Table 3). Intake of silage fat decreased with plant maturity and with concentrate supplementation ($P < 0.001$), and intake of dietary ME decreased with increasing plant maturity and increased with concentrate supplementation ($P < 0.001$). Age at slaughter increased with increasing plant maturity and decreased with concentrate supplementation ($P < 0.001$). Daily cold carcass gain reached a maximum of 832 and 814 g/day when silage H2 and H1 were fed with concentrates, respectively. Because all bulls were slaughtered close to target LW, their age at slaughter followed daily LWG. Bulls offered the poorest diet (H3 silage as sole feed) were nearly 18 months old at slaughter, whereas the fastest-growing bulls were 14 months old.

Table 2. Fatty acid profile [a], g/100 g fatty acid, and concentrations of fatty acids[b], g/kg DM, of silages and concentrate.

	Fatty acid profile of silage and concentrate, g/100 g fatty acid								Concentrations of fatty acids, g/kg DM			
	Harvesting time for silage [h]								Harvesting time for silage			
	H1		H2		H3		Concentrate		H1	H2	H3	Concentrate
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.				
C12:0	0.12	0.014	0.15	0.007	0.21	0.007	0.06	0.000	0.04	0.04	0.05	0.02
C14:0	0.36	0.028	0.42	0.007	0.58	0.007	0.50	0.028	0.12	0.11	0.13	0.19
C15:0	0.09	0.014	0.09	0.014	0.18	0.000	0.11	0.007	0.03	0.02	0.04	0.04
C16:0	14.1	0.45	14.7	0.21	15.8	0.29	23.4	0.276	4.75	3.85	3.53	9.23
C16:1 c9	0.43	0.000	0.72	0.028	0.64	0.247	0.32	0.014	0.14	0.19	0.14	0.13
C17:0	0.10	0.007	0.10	0.007	0.15	0.035	0.13	0.007	0.03	0.03	0.03	0.05
C18:0	1.25	0.028	1.22	0.021	1.52	0.014	2.53	0.035	0.43	0.32	0.34	1.01
C18:1 c9	2.72	0.014	2.80	0.028	3.27	0.057	34.94	0.113	0.93	0.74	0.74	13.93
C18:2 n-6	15.9	0.28	15.3	0.14	15.3	0.17	25.2	0.007	5.41	4.05	3.45	10.04
C18:3 n-3	48.9	0.40	47.3	0.58	42.7	1.17	0.75	0.007	16.64	12.52	9.63	0.30
SFA[c]	16.1	0.47	16.8	0.22	18.5	0.34	26.9	0.36	5.40	4.37	4.12	10.55
MUFA[d]	3.2	0.01	3.5	0.000	3.9	0.30	35.3	0.10	1.07	0.93	0.88	14.06
PUFA[e]	64.8	0.67	62.6	0.44	58.0	1.34	26.0	0.000	22.05	16.57	13.09	10.34
n-6/n-3[f]	0.33	0.003	0.32	0.007	0.36	0.006	33.9	0.33	0.33	0.32	0.36	33.6
Total identified acids[g]	84.0	0.22	82.8	0.22	80.4	0.70	88.1	0.26	28.5	21.9	18.1	34.9
Not identified	16.0	0.22	17.2	0.22	19.6	0.70	11.9	0.26	5.39	4.50	4.38	4.69

N = 2 (one sample analyzed in duplicate) for fatty acid profile. Mean values within harvests are used for concentrations of individual fatty acids in g/kg DM.
[a]g/100 g fatty acid methyl esters (FAME).
[b]Fatty acid profile converted to concentrations in g/kg DM by using crude fat concentrations of feeds (Table 1) and conversion factors for individual fatty acids from 0.856 for C12:0–0.897 for C18:0, based on molecular weights for each fatty acid, and glycerol.
[c]Saturated: C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0.
[d]Monounsaturated: C16:1c9, C18:1c9.
[e]Polyunsaturated: C18:2n-6, C18:3n-3.
[f]C18:2n-6/C18:3n-3.
[g]Including C8:0 and C10:0, which are omitted from the table because all values were <0.1 g/100 g fatty acid.
[h]Maturity stage of timothy, the dominating grass species at harvest, given as mean stage by weight (MSW) was 2.44, 2.73 and 3.30, for H1, H2 and H3, respectively (Moore et al., 1991).

Table 3. Effect of harvesting time for silage and concentrate supplementation on feed intake and daily gain of bulls, carcass assessments, and intramuscular fat and α-tocopherol concentrations in *M. longissimus thoracis*.

	Harvesting time for silage [d]									
	H1		H2		H3			*P*-value[g]		
	No conc.[e]	With conc.[f]	No conc.	With conc.	No conc.	With conc.	SEM	Harvest time	Conc.	Interaction[h]
Daily feed intake										
Silage DM, kg	9.21	7.34	8.92	6.92	7.79	6.90	0.204	<0.001	<0.001	0.02
Concentrate DM, kg		2.67		2.63		2.61				
Silage fat, g	350	280	264	205	197	174	6.80	<0.001	<0.001	0.005
Concentrate fat, g		109		107		107				
Dietary metabolisable energy, MJ	104.7	114.3	95.1	104.3	75.0	96.6	2.19	<0.001	<0.001	0.01
Age of bulls and daily weight gain										
Age at slaughter, d	450	427	466	432	543	454	10.2	<0.001	<0.001	0.007
Live weight at slaughter, kg	572	572	568	577	572	573	2.98	0.97	0.17	0.28
Daily cold carcass gain, g	735	814	646	832	451	695	21.6	<0.001	<0.001	0.003
Carcass assessments										
Cold carcass weight, kg	297	296	291	301	282	293	3.76	0.09	0.06	0.30
Dressing proportion[a]	519	518	511	521	494	511	5.35	0.04	0.08	0.28
Carcass conformation[b]	5.6	5.6	5.4	5.3	5.2	5.7	0.20	0.45	0.44	0.37
Carcass fat class[c]	8.1	7.9	6.7	6.9	5.7	7.0	0.48	0.02	0.33	0.26
Dissected fat, g/kg carcass	148	163	141	134	108	138	8.60	0.02	0.10	0.16
In *Musculus longissimus thoracis*										
Intramuscular fat, g/100 g muscle	2.32	3.02	2.03	2.33	2.24	2.15	0.199	0.03	0.07	0.16
α-tocopherol, mg/100 g muscle	0.19	0.21	0.14	0.13	0.14	0.20	0.021	0.02	0.20	0.22
α -tocopherol, mg/100 g fat	9.09	7.01	7.49	5.79	6.10	9.55	1.08	0.39	0.90	0.03

[a]Cold carcass weight, gram per kg of live weight at slaughter.
[b]EUROP-classification scores transformed to scale 1–15, where 1 is *P*- and 15 is E+. *P*- (transformed to 1) is the leanest (poorest) conformation and E+ (transformed to 15) is the most swelling (best).
[c]Carcass fat classification, where the degree of fat is denoted by the numbers 1, 2, 3, 4, 5 in order of increasing fatness, and added minus, nothing or plus. Fatness scores were transformed to scale 1–15, where 1 is 1- and 15 is 5 + .
[d]Maturity stage of timothy, the dominating grass species at harvest, given as mean stage by weight (MSW) was 2.44, 2.73 and 3.30, for H1, H2 and H3, respectively (Moore et al., 1991).
[e]Bulls fed grass silage *ad libitum* and supplemented with mineral and vitamin mixture, only.
[f]Bulls fed grass silage *ad libitum* and supplemented with mineral and vitamin mixture, and 2 - 4 kg concentrate daily at increasing live weight.
[g]*P*-value < 0.05 is considered significant. *P*-value ≥ 0.05 and < 0.10 is considered to indicate a trend. *P*-value ≥ 0.10 is considered not significant.
[h]Harvest time × concentrate supplementation interaction.

Carcass assessments, and concentrations of IMF and α-tocopherol in LT

Cold carcass weight and carcass conformation did not differ significantly among treatments. All bulls were assigned a conformation score O or O+. Dressing proportion, carcass fat classification, and dissected fat proportion decreased with increasing plant maturity ($P = 0.04$, $P = 0.02$, and $P = 0.02$, respectively; Table 3). Cold carcass weight and dressing proportion tended to increase with concentrate supplementation ($P = 0.06$ and $P = 0.08$, respectively).

Intramuscular fat concentration in LT decreased with increasing plant maturity ($P = 0.03$) and tended to increase with concentrate supplementation ($P = 0.07$). The α-tocopherol concentration given in mg/100 g of LT decreased with increasing plant maturity ($P < 0.02$). Due to the confounding effect of α-tocopheryl acetate supplementation, where concentrate fed animals received on average 1.0 mg daily with the concentrate, in addition to 0.2 mg daily from the mineral and vitamin mix offered all animals, α-tocopherol concentrations should be evaluated separately for bulls fed without or with concentrate. For silage-only bulls, α-tocopherol-concentration, mg/100 g muscle, decreased with increasing plant maturity ($P = 0.049$) with a similar tendency ($P < 0.09$) for concentrate fed bulls (not presented in Table). No differences between plant maturity stages were found for α-tocopherol concentration in mg/100 g IMF within silage-only bulls. However, a tendency was observed ($P = 0.06$; not presented in Table) for increased α-tocopherol concentration with increasing plant maturity for supplemented bulls, where only the difference between H2 and H3 (5.79 vs. 9.55 mg/100 g IMF; Table 3) was significant ($P = 0.02$).

Fatty acid profile of IMF, and concentrations of some fatty acids in muscle

Dietary treatments influenced neither the proportions of the most abundant individual saturated fatty acids (SFA), C16:0, C18:0, or C14:0, nor the sum of SFA in IMF from LT (Table 4). The minor SFA, C17:0, C15:0, and C20:0, all increased with increasing plant maturity ($P < 0.001$, $P < 0.001$ and $P < 0.009$, respectively), and C17:0 and C15:0 decreased with concentrate supplementation ($P < 0.001$).

Table 4. Effect of harvesting time for silage and concentrate supplementation on fatty acid profile of intramuscular fat of *Musculus longissimus thoracis*, g/100 g fatty acids.

	Harvesting time for silage[h]									
	H1		H2		H3		*P*-value [k]			
	No conc.[i]	With conc. [j]	No conc.	With conc.	No conc.	With conc.	SEM	Harvest time	Conc.	Interaction [m]
C14:0 [g]	2.40	1.96	2.12	2.10	2.23	1.99	0.159	0.88	0.05	0.31
C14:1 c9 [g]	0.59	0.52	0.54	0.52	0.45	0.46	0.082	0.41	0.63	0.88
C15:0	0.36	0.24	0.39	0.30	0.57	0.34	0.020	<0.001	<0.001	0.003
C15:1 [a g]	0.11	0.09	0.14	0.10	0.16	0.11	0.009	0.002	<0.001	0.06
C16:0 [g]	24.64	23.30	23.60	24.09	24.31	23.88	0.679	0.91	0.39	0.29
C16:1 c9	3.36	3.66	3.24	3.24	3.13	3.11	0.218	0.20	0.61	0.71
C17:0	0.80	0.67	0.93	0.76	1.11	0.80	0.041	<0.001	<0.001	0.09
C17:1 [a g]	0.88	0.80	1.08	0.77	1.02	0.88	0.038	0.01	<0.001	0.003
C18:0	13.53	13.70	13.34	14.98	14.43	14.64	0.642	0.37	0.21	0.44
C18:1 t9	0.38	0.39	0.36	0.41	0.38	0.44	0.021	0.41	0.03	0.48
C18:1 t11 (TVA) [g]	1.24	0.67	1.18	0.95	1.50	1.08	0.131	0.02	<0.001	0.31
C18:1 c9	33.48	37.81	33.82	35.47	31.62	34.65	0.622	0.002	<0.001	0.12
C18:1 c11	1.15	1.27	1.21	1.17	1.19	1.28	0.052	0.72	0.23	0.29
C18:2 t9t12 n-6 [g]	0.23	0.18	0.20	0.16	0.16	0.13	0.012	<0.001	<0.001	0.56
C18:2 c9t11 (CLA) [g]	0.22	0.12	0.22	0.17	0.20	0.17	0.029	0.61	0.01	0.44
C18:2 c9c12 n-6 [g]	2.55	3.61	2.63	3.47	2.80	3.98	0.301	0.35	<0.001	0.79
C18:3 c9c12c15 n-3 (ALA) [g]	1.79	1.37	1.69	1.10	1.54	1.02	0.123	0.04	<0.001	0.69
C20:0	0.09	0.08	0.09	0.10	0.10	0.11	0.005	0.009	0.39	0.24
C20:3 n-6 (DGLA) [g]	0.19	0.25	0.21	0.23	0.21	0.28	0.024	0.41	0.008	0.58
C20:4 n-6 (ARA) [g]	1.17	1.33	1.28	1.19	1.38	1.54	0.136	0.11	0.44	0.48
C20:4 n-3 (ETA) [g]	0.17	0.13	0.18	0.10	0.19	0.10	0.014	0.57	<0.001	0.10
C20:5 n-3 (EPA) [g]	0.77	0.59	0.84	0.48	0.86	0.49	0.055	0.87	<0.001	0.11
C22:5 n-3 (DPA) [g]	1.07	0.98	1.15	0.87	1.11	0.89	0.072	0.95	0.001	0.31
C22:6 n-3 (DHA) [g]	0.14	0.12	0.18	0.13	0.17	0.15	0.018	0.22	0.02	0.34
Total acids identified [g]	91.3	92.7	91.2	92.9	91.0	92.9	0.343	0.96	<0.001	0.72
SFA [b g]	41.8	39.8	40.5	42.3	42.8	41.8	0.918	0.22	0.56	0.05
MUFA [c g]	41.2	44.2	42.1	42.6	39.6	42.3	0.853	0.07	0.003	0.20
PUFA [d g]	8.3	8.7	8.6	7.9	8.6	8.7	0.627	0.69	0.91	0.59
C18:2c9,12 n-6/C18:3c9,12,15 n-3	1.45	2.63	1.65	3.16	1.84	3.81	0.117	<0.001	<0.001	0.009
n-6 PUFA[e g]	3.91	5.19	4.11	4.90	4.39	5.80	0.440	0.23	0.001	0.68
n-3 PUFA[f g]	3.94	3.20	4.03	2.67	3.88	2.64	0.226	0.34	<0.001	0.26
n-6/n-3 PUFA	1.00	1.73	1.06	1.84	1.14	2.10	0.053	<0.001	<0.001	0.09

TVA Trans-vaccenic acid; CLA Conjugated linoleic acid; ALA α-linolenic acid; DGLA Dihomogammalinoleic acid; ARA Arachidonic acid; ETA Eicosatetraenoic acid; EPA Eicosapentaenoic acid; DPA Docosapentaenoic acid; DHA docosahexaenoic acid; SFA Saturated fatty acids; MUFA Monounsaturated fatty acids; PUFA polyunsaturated fatty acids.

[a]The sum of cis9, cis10 and other isomers.

[b]Saturated: C14:0+C15:0+C16:0+C17:0+C18:0+C20:0.

[c]Monounsaturated: C14:1c9+C15:1+C16:1c9+C17:1+C18:1t9+C18:1t11+C18:1c9+C18:1c11.

[d]Polyunsaturated: all analyzed C18, C20 and C22-acids with more than one double bond.

[e]C18:2c9c12 n-6+C20:3 n-6+C20:4 n-6.

[f]C18:3c9c12c15 n-3+C20:4 n-3+C20:5 n-3+C22:5 n-3+C22:6 n-3.

[g]Values are covariance corrected for intramuscular fat concentration.

[h]Maturity stage of timothy, the dominating grass species at harvest, given as mean stage by weight (MSW) was 2.44, 2.73 and 3.30, for H1, H2 and H3, respectively (Moore et al., 1991).

[i]Bulls fed grass silage *ad libitum* and supplemented with mineral and vitamin mixture, only.

[j]Bulls fed grass silage *ad libitum* and supplemented with mineral and vitamin mixture, and 2 - 4 kg concentrate daily at increasing live weight.

[k]*P*-value < 0.05 is considered significant. *P*-value ≥ 0.05 and < 0.10 is considered to indicate a trend. *P*-value ≥ 0.10 is considered not significant.

[m]Harvest time × concentrate supplementation interaction.

The proportions of the overall most abundant fatty acid in IMF of beef, C18:1c9, and also the sum of monounsaturated fatty acids (MUFA), decreased ($P = 0.002$) or tended to decrease ($P = 0.07$) with increasing plant maturity, respectively, and increased with concentrate supplementation ($P < 0.001$ and $P < 0.003$, respectively; Table 4). The proportions of the odd-chained MUFA C17:1 and C15:1 increased with increasing plant maturity ($P < 0.01$ and $P < 0.002$, respectively) and decreased with concentrate supplementation ($P < 0.001$). A similar pattern was found for C18:1t11 ($P < 0.02$ and $P < 0.001$, respectively).

Of PUFA, C18:2t9t12 n-6 and C18:3 n-3 proportionally decreased with increasing plant maturity ($P < 0.001$ and $P < 0.04$, respectively) whereas all other PUFA were unaffected by plant maturity (Table 4). C18:2 n-6 and C20:3 n-6 increased with concentrate supplementation ($P < 0.001$ and $P < 0.008$, respectively), and C20:4 n-6 was unaffected, whereas all other PUFA decreased with concentrate supplementation ($P < 0.02$ for C22:6 n-3, $P = 0.001$ for C22:5 n-3, otherwise $P < 0.001$). The n-6/n-3 ratio for C18 PUFA, and for all PUFA, increased with increasing plant maturity and with concentrate supplementation ($P < 0.001$). The P/S ratio (PUFA/SFA) averaged 0.21 and

Table 5. Effect of harvesting time for silage and concentrate supplementation on fatty acid contents of intramuscular fat of *Musculus longissimus thoracis* in mg/100 g muscle.

	Harvesting time for silage[c]									
	H1		H2		H3		*P*-value [f]			
	No conc. [d]	With conc.[e]	No conc.	With conc.	No conc.	With conc.	SEM	Harvest time	Conc.	Interaction[g]
C16:0	530	677	433	516	497	470	53.2	0.04	0.13	0.27
C18:0	286	380	247	319	298	289	28.8	0.21	0.04	0.20
C18:1 t11 (TVA)	26.9	23.6	20.8	19.9	30.9	21.0	4.14	0.35	0.18	0.54
C18:1 c9	716	1051	629	764	650	687	68.8	0.009	0.006	0.11
C18:2 c9t11 (CLA)	4.72	4.74	3.78	3.67	4.12	3.27	0.870	0.41	0.66	0.86
C18:2 c9c12 n-6	53.2	79.5	52.8	73.1	57.9	77.6	4.53	0.56	<0.001	0.72
C18:3 c9c12c15 n-3 (ALA)	37.8	30.4	32.4	23.2	31.6	20.7	2.54	0.01	<0.001	0.78
C20:3 n-6 (DGLA)	3.76	4.84	4.34	4.81	4.40	5.42	0.318	0.18	0.003	0.58
C20:4 n-6 (ARA)	23.4	24.4	26.3	25.1	28.7	30.1	1.60	0.007	0.74	0.67
C20:4 n-3 (ETA)	3.43	2.32	3.57	2.18	4.00	2.02	0.233	0.79	<0.001	0.19
C20:5 n-3 (EPA)	15.4	10.1	16.5	10.1	17.8	10.2	0.825	0.34	<0.001	0.39
C22:5 n-3 (DPA)	21.4	17.7	23.0	18.3	23.1	18.1	1.11	0.52	<0.001	0.83
C22:6 n-3 (DHA)	2.68	2.29	3.58	2.63	3.54	2.88	0.247	0.01	0.003	0.55
Long chain n-6 PUFA[a]	27.1	29.3	30.6	29.9	33.1	35.6	1.88	0.01	0.41	0.64
Long chain n-3 PUFA[b]	42.9	32.4	46.7	33.2	48.4	33.3	2.13	0.31	<0.001	0.55

TVA trans vaccenic acid; CLA conjugated linoleic acid; ALA α-linolenic acid; DGLA Dihomogammalinoleic acid; ARA Arachidonic acid; ETA Eicosatetraenoic acid; EPA Eicosapentaenoic acid; DPA Docosapentaenoic acid; DHA docosahexaenoic acid; PUFA polyunsaturated fatty acids.

[a]C20:3 n-6+C20:4 n-6.

[b]C20:4 n-3+C20:5 n-3+C22:5 n-3+C22:6 n-3.

[c]Maturity stage of timothy, the dominating grass species at harvest, given as mean stage by weight (MSW) was 2.44, 2.73 and 3.30, for H1, H2 and H3, respectively (Moore et al., 1991).

[d]Bulls fed grass silage *ad libitum* and supplemented with mineral and vitamin mixture, only.

[e]Bulls fed grass silage *ad libitum* and supplemented with mineral and vitamin mixture, and 2–4 kg concentrate daily at increasing live weight.

[f]*P*-value < 0.05 is considered significant. *P*-value ≥ 0.05 and < 0.10 is considered to indicate a trend. *P*-value ≥ 0.10 is considered not significant.

[g]Harvest time × concentrate supplementation interaction.

was not affected by plant maturity stage or concentrate supplementation (not presented in Table).

Increasing plant maturity decreased the amounts of C16:0, C18:1c9, and C18:3 n-3 measured in mg/100 g muscle of LT ($P < 0.04$, $P < 0.009$ and $P < 0.01$, respectively), while the amounts of C20:4 n-6, C22:6 n-3, and the sum of long-chain n-6 PUFA ($P < 0.007$, $P < 0.01$ and $P < 0.01$, respectively) increased (Table 5). Concentrate supplementation increased the amounts of C18:0 and C18:1c9 ($P < 0.04$ and $P < 0.006$, respectively) in mg/100 g muscle. Apart from C20:4 n-6, that was unaffected by concentrate supplementation, the two other n-6 acids increased ($P < 0.001$ for C18:2 n-6 and $P < 0.003$ for C20:3 n-6), and all n-3 acids decreased ($P < 0.003$ for C22:6 n-3, otherwise $P < 0.001$) by concentrate supplementation. The long-chain PUFA n-6/n-3 ratio in muscle increased with increasing plant maturity and with concentrate supplementation ($P < 0.008$ and $P < 0.001$, respectively, not presented in Table). In mg/100 g muscle, neither C18:1t11 nor C18:2c9t11 were influenced by plant maturity or concentrate supplementation (Table 5).

Discussion

Feed composition

Forages provide substantial lipids in ruminant diets (Harfoot & Hazelwood, 1988). Exploiting the potential of herbage as an alternative to marine sources of PUFA is an important nutritional strategy for enhancing the content of n-3 PUFA in beef (Scollan et al., 2006). In regions with short growth periods, PUFA from forage represent a low-cost approach in comparison with diet supplementation strategies, and it provides beef consumers with dietary n-3 PUFA without competition with marine sources. Esterified lipids in forages represent two-thirds of the total lipids, and are composed of approximately 50% galactolipids, 33% simple lipids (diglycerides, free fatty acids, waxes and sterol esters) and 17% phospholipids (Bauchart et al., 1985). The proportions of the three most abundant fatty acids in the silages, as means over the three harvesting times, were 46 g C18:3 n-3, 16 g C18:2 n-6 and 15 g C16:0 per 100 g of fatty acid, which were similar to other results with timothy (Boufaied et al., 2003) and mixed timothy and meadow fescue sward (Halmemies-Beauchet-Filleau et al., 2013). The observation that the proportion of C18:3 n-3 decreased with increasing plant maturity, whereas proportions of all other identified acids increased with increasing maturity was consistent with Boufaied et al. (2003), apart from a decreasing proportion of C18:2 n-6 with increasing maturity in their study. Because plant lipids are mainly located in the chloroplasts of plant leaves, fat concentration in forages decreases when leaf/stem proportions decrease with increasing plant maturity (Boufaied et al., 2003) as observed in the present study.

Concentrations of IMF and α-tocopherol in LT

Concentrate inclusion tended to increase IMF content in LT, in line with other studies where concentrates increased energy intake (Keady et al., 2007; Faucitano et al., 2008). Vitamin E is a powerful antioxidant with several beneficial health effects for humans and animals. Vitamin E protects against infections (Hogan et al., 1993), may contribute to depress development of cancer, and may prevent or delay coronary heart disease (Daley et al., 2010). Also, vitamin E maintains the red colour (oxymyoglobin) compared with the brown, oxidised metmyoglobin of beef (McDowell et al., 1996). Although grass feeding provides animals with high proportions of PUFA, it improves shelf life of meat due to its high content of vitamin E (Scollan et al., 2006). The observed α-tocopherol levels in LT were similar to those found in forage-fed cattle by Marino et al. (2006) and Eriksson and Pickova (2007) where meat was aged for 14 days, but lower than 3.1–3.9 mg/kg meat found in other studies with silage or pasture-fed cattle where meat was immediately frozen (Realini et al., 2004; Warren et al., 2008b). Richardson et al. (2005) and Warren et al. (2008b) found considerably higher vitamin E concentrations in beef from silage-fed compared with concentrate-fed steers. In the present study, concentrate supplementation did not reduce α-tocopherol concentrations in LT or in IMF, possibly because the concentrate, as well as the mineral and vitamin mixture, provided bulls with vitamin E supplement: 130 I.U. (concentrate-supplemented bulls) and 200 I.U. (all bulls) per head daily, respectively. Whereas the highest meat α-tocopherol concentrations in this study might provide oxidative stability of meat, a 100 g daily portion would provide only about 1-2% of the recommended dietary allowance for humans, that is 8–10 mg (Nordic Nutrition Recommendations, 2012).

Fatty acid profile of IMF, and fatty acid concentrations in muscle

Intramuscular fat mainly consists of triacylglycerols and phospholipids (Scollan et al., 2006). The phospholipid is an essential component of cell membranes, and its amount remains fairly constant, or increases slightly, as the animal increases in fatness (Wood et al., 2008). Beef meat with an IMF content of 2–5%, like in the present study, is considered as low in fat, and characterised as lean (Wood et al., 2008). In lean animals, the proportionally lower C18:1c9 and higher C18:2 n-6 content of phospholipid compared with neutral lipid have a major influence on total muscle fatty acid composition (Wood et al., 2008). In lean, 14-months old steers with a fatness score of 54.9, the phospholipid fraction constituted 0.3 of total IMF lipids, in contrast to only 0.07 in 24-months old, fat steers with a fatness score of 107 (Warren et al., 2008a). In the present study, the proportion of total SFA in IMF was in the same range as for pasture fed (Realini et al., 2004) or grass silage fed (Warren et al., 2008a) steers, but below the range 0.45–0.48 given by Scollan et al. (2006). Apart from C20:0, which constituted only 0.001 of total fatty acids in IMF, and increased with increasing plant maturity, none of the even chain individual SFA was influenced by plant maturity stage, although they proportionally increased with increasing maturity stage in the offered silage. Daily intake of C16:0 and C18:0 acids, however, was higher in bulls fed H1 silage than H2 and H3 silage because silage fat concentration, as well as silage DM intake were higher. The proportion in IMF of the most prevalent MUFA, C18:1c9, was similar to that found with forage feeding by Warren et al. (2008a) and Realini et al. (2004), and decreased with increasing plant maturity. This was probably caused by a decrease in IMF, as well, leading to a proportional increase in the phospholipid fraction. In the fatty acid profile, C18:1c9 was positively correlated to IMF (Adjusted $R^2 = 0.28$, N = 36, $P < 0.001$), and C18:2 n-6 negatively correlated to IMF (Adjusted $R^2 = 0.27$, N = 36, $P < 0.001$). Additionally, C18:3 n-3, which is the major fatty acid in grass and the precursor of the n-3 series of long-chain fatty acids, decreased in silage with increasing plant maturity, proportionally and as daily dietary supply (Table 2). The proportion of C18:3 n-3 was negatively correlated to IMF concentration (Adjusted $R^2 = 0.23$, N = 36, $P = 0.002$). Because fibrous forage has a long rumen transit time, fat in forage is extensively hydrogenated, which may limit its incorporation into adipose tissue and muscle compared with C18:2 n-6 from concentrates (Wood et al., 2008).

The major trans fatty acid in beef, C18:1t11, was found in similar proportions as in silage-fed steers by Warren et al. (2008a) and increased with increasing plant maturity. Its desaturase product, C18:2c9t11, was also found in similar proportions as by Warren et al. (2008a) but was not influenced by plant maturity. Realini et al. (2004) found 0.41 g of C18:2c9t11 per 100 g IMF in pasture-finished steers with 1.68% IMF, and 0.23 g in concentrate-finished steers with 3.18% IMF. On a muscle basis, C18:2c9t11 concentrations must have been similar for the two diets, and somewhat higher than the average value 4.0 mg of C18:2c9t11 per 100 g muscle found in the present study. This is the major CLA isomer in ruminant products, and is mainly associated with the neutral lipid fraction, and therefore

positively correlated with IMF concentration (Scollan et al., 2006). In the present study, only a weak and insignificant positive relationship was found between IMF and C18:2c9t11 proportion in IMF. However, significant positive relationships were found between IMF and C18:2c9t11 given in mg/100 g muscle, with adjusted $R^2 = 0.51$, $N = 36$, $P < 0.001$, and also between IMF and C18:1t11, with adjusted $R^2 = 0.36$, $P < 0.001$. Significant positive relationships were also found between IMF and C18:2 n-6 and C18:3 n-3 in mg/100 g muscle, $R^2 = 0.12$ and 0.11, respectively, $P = 0.02$ and $P = 0.03$, respectively. Whereas C20:3 n-6 and C20:4 n-3, in mg/100 g muscle, were not related to IMF, the other long-chain PUFA, including the sums of long-chain n-6 PUFA and long-chain n-3 PUFA, were all, when given in mg/100 g muscle, significantly negatively related to IMF, with adjusted R^2 between 0.10 and 0.17.

Odd- and branched-chain fatty acids only occur at trace levels in plants, however with the rumen bacteria as its major cause, these fatty acids are distinct components of milk and adipose tissue in ruminants (Vlaeminck et al., 2006). Higher milk secretion than the duodenal flow of C15:0 and C17:0 suggests *de-novo* synthesis from propionate in animal tissue, and C17:1 is suggested to be a desaturase product of C17:0 (Vlaeminck et al., 2006). The reason why proportions of these odd- and branched-chain fatty acids, including C15:1, in IMF, increased significantly with increasing plant maturity and decreased with concentrate supplementation in the present study is unknown. However, Vlaeminck et al. (2006) found higher proportions of milk odd- and branched-chain fatty acids with increasing dietary forage to concentrate ratio, similar to higher levels in forage only-diets in the present study. They proposed that changes in milk concentrations of iso-fatty acids and *anteiso* C15:0 might reflect differences in the rumen bacterial populations induced by variation in the dietary forage to concentrate ratio.

The n-6/n-3 ratio at the C18 chain length was at the same level as found by Moloney et al. (2013) in heifers finished at a similar diet, and the total n-6/n-3 ratio over all chain lengths (C18, C20 and C22) were at the same low level as found for pasture-derived label beef of calves, heifers and steers by Razminowicz et al. (2006) and in forage-fed steers by Turner et al. (2011). Significantly lower proportions of long-chain n-6 PUFA ($P = 0.03$), as well as long-chain n-3 PUFA ($P = 0.01$), with increasing IMF on muscle basis might be caused by the higher phospholipid proportions in IMF in leaner bulls (Wood et al., 2008). This might partly explain the increasing total n-6/n-3 ratio with increasing plant maturity ($P < 0.001$; Table 4) that gave leaner bulls ($P = 0.02$; Table 3), however, there was no direct relationship between total PUFA n-6/n-3 ratio and IMF concentration.

The n-6/n-3 ratio (Table 4) was down to 1.0 for bulls offered solely grass silage from the earliest harvesting time. Whereas a healthy human diet consists of one to four times more n-6 than n-3 fatty acids, typical diets may contain up to 30 times more n-6 (Daley et al., 2010). Although the human body may convert C18:2 n-6 and C18:3 n-3 to long-chain PUFA, the conversion is below 5% in humans, so the majority of these long-chain fatty acids that are used by the body, especially C20:5 n-3, C22:5 n-3, and C22:6 n-3, is consumed in the diet (Daley et al., 2010). None of the studies reviewed by Scollan et al. (2006) or Daley et al. (2010) found similar low n-6/n-3 ratios, apart from one study where protected fish oil was included in the diet. Faucitano et al. (2008) observed an n-6/n-3 ratio of 1.2 when Angus cross steers were offered solely grass silage, and French et al. (2000) 2.3 when continental crossbred steers were offered grazed grass, only. The most abundant long-chain n-3 PUFA in this study was C22:5 n-3. As reviewed by McAfee et al. (2010), red meat is the main human dietary source of C22:5 n-3. It accumulates in mammals but not in oily fish and is suggested to have beneficial health effects (Kaur et al., 2011; Richter et al., 2019), however, research stating this is still lacking (Clayton, 2014).

The European Food Safety Authority (EFSA, 2010) recommends a daily adequate intake (AI) of 250 mg for C20:5 n-3 plus C22:6 n-3. For a food to be classified as 'a source' or 'high in' C20:5 n-3 plus C22:6 n-3, a normal daily portion must provide 15 or 30% of recommended AI, that is 40 or 80 mg, respectively (Commission Regulation (EU) No 116/2010). Daily human consumption of 100 g beef from the current study would provide on average 20 or 13 mg, if bulls were offered solely grass silage or were concentrate supplemented, respectively, that is too little to claim the beef as 'a source' of C20:5 n-3 plus C22:6 n-3. However, other and fatter tissues, e.g. adipose tissue, are frequently included in minced meat products. Fatty acid composition of other tissues is also influenced by diet and therefore broadly similar to that of the longissimus muscle (Wood et al., 2008). Carvalho and Smith (2018) found significant positive correlations between subcutaneous adipose tissue and *M. longissimus dorsi* muscle for concentrations of all studied fatty acids. A 100-g daily portion of sausages with 15% fat, with the same composition as with solely grass silage-fed beef in the present study would provide 150 mg C20:5 n-3 plus C22:6 n-3, and with diets including concentrate, 85 mg. However, the Commission Regulation (EU) No 116/

2010 of the European Union states that for a food to be claimed as 'a source' or being 'high in' C20:5 n-3 plus C22:6 n-3, it should as well provide 40 or 80 mg of C20:5 n-3 plus C22:6 n-3, respectively, per 100 kcal product. Assuming an energy value of 210 kcal per 100 g sausage (www.matvaretabellen.no; The Norwegian Food Safety Authority, 2021), the portions would provide 71 and 40 mg of C20:5 n-3 plus C22:6 n-3 per 100 kcal sausage if bulls were solely grass silage-fed, or concentrate supplemented, respectively, and both could be claimed as 'a source' of C20:5 n-3 plus C22:6 n-3. Both these 100-g portions would provide 6.3 g of SFA. According to EFSA (2010), SFA intake should be as low as is possible within a nutritionally adequate diet.

The decreased proportions of C18:3 n-3 and all its long-chain PUFA n-3 products, which resulted in increased n-6/n-3 proportion with concentrate supplementation, are typical alterations in fatty acid composition observed in studies where forage based and concentrate based diets are compared (Realini et al., 2004; Scollan et al., 2006; Faucitano et al., 2008; Warren et al., 2008a; Daley et al., 2010). Also, Siphambili et al. (2020) found higher LT muscle concentrations of all the four long-chain n-3 PUFA when bulls were finished on pasture only, compared with indoors on concentrates, although significance was reached only for C20:5 n-3 and C22:6 n-3. Increased proportion of C18:1c9 with concentrate feeding was also observed by Realini et al. (2004) and Faucitano et al. (2008), and increased C18:2 n-6 by Warren et al. (2008a). These changes, however, were not general for concentrate versus forage feeding, and may depend on differences in concentrate type and level, and particularly how concentrate feeding affects animal fatness at slaughter (Wood et al., 2008). In the present study, increased C18:1c9 proportion in IMF with concentrate feeding was consistent both with a higher dietary supply of C18:1c9 and with a tendency of concentrate to increase IMF concentration. The observed increase in C18:2 n-6 proportion in IMF with concentrate feeding might have been caused by increased dietary supply. In spite of increased C18:2 n-6 proportion in feed, and increased IMF in concentrate supplemented bulls, its longer chain derivative C20:4n-6 did not increase in IMF of these bulls, as previously observed (Scollan et al., 2006).

Conclusions

Bulls offered silage from grass crops harvested at a very early maturity stage, with tillers in stem elongation without visible heads, produced beef with the lowest n-6/n-3 proportions in IMF, in line with the hypothesis. Such immature grass crops contain more fat, and fat with a higher proportion of C18:3 n-3 than more mature grass crops that are commonly used for silage production. Concentrate supplementation increased proportions of C18:1c9, C18:2 n-6, and C20:3 n-6, and decreased the proportions of C18:1t11, C18:2c9t11, C18:3 n-3, and all the long-chain n-3 acids. This resulted in beef with higher n-6/n-3 ratio in the fatty acid profile of IMF compared with unsupplemented bulls, in line with the hypothesis. Results suggest that 'grass-fed beef' with a fatty acid profile that is beneficial for human health, with an n-6/n-3 ratio down to 1.0, commonly produced in pasture-based systems, also may be produced indoors on grass silage in regions with short grazing seasons.

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ORCID

Åshild T. Randby http://orcid.org/0000-0003-0674-9269

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