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Addition of an extract of lucerne (*Medicago sativa* L.) to cattle diets – effects on fatty acid profile, meat quality and eating quality of the *M. Longissimus* muscle.

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Abstract

There is considerable interest in enhancing beneficial fatty acids, particularly 18:3 n -3, conjugated linoleic acid and long chain n -3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in beef to provide benefits to human health. Here, the potential to enhance these fatty acids by feeding a n -3 polyunsaturated fatty acid (PUFA)-rich plant extract (PX) from lucerne is presented. Cattle ($n=8$ /diet) were assigned to the following finishing diets: straw and concentrate (S-CC); straw and concentrate containing 25% PX and 2000 IU vitamin E/kg PX-concentrate (S-PXC); grass silage (GS); GS plus 75 g PX/kg dry matter intake (DMI) (GS-LPX) or GS plus 150 g PX/kg DMI (GS-HPX). Addition of PX to concentrates or GS increased the n -3 fatty acid content of loin muscle and had favourable effects on the n -6: n -3 PUFA ratio ($P < 0.05$), without detrimental effects on loin muscle sensory characteristics.

Keywords: beef; PUFA; lucerne; forage; biohydrogenation.

1. Introduction

Consumers are increasingly concerned about the nutritional and health value of their food (Nikolova & Inman, 2015). Despite beef being recognised as an important source of protein and micronutrients, such as vitamins A, B₆, B₁₂, D, iron, selenium and zinc, its popularity as a food choice has been adversely affected by the perception that beef contains high amounts of fat, in particular saturated fatty acids (SFA), and thus has been associated with cardiovascular disease and cancer (McAfee, et al., 2010; Williams, 2007). Human health concerns about excessive consumption of SFA are well documented, and there is evidence to suggest that replacing SFA with polyunsaturated fatty acids (PUFA), in particular *n*-3 PUFA, may provide additional health benefits, as opposed to substituting with carbohydrates (FAO, 2010; Siri-Tarino, Sun, Hu, & Krauss, 2010). Specifically, the long chain *n*-3 fatty acids eicosapentaenoic acid (EPA; 20:5*n*-3) and docosahexaenoic acid (DHA; 22:6*n*-3) have recognised benefits to human health (Barceló-Coblijn & Murphy, 2009; Calder, 2004; Lopez-Huertas, 2010; Russo, 2009; Salter, 2013). The daily requirements for EPA+DHA or 18:3*n*-3 in the human diet are indicated to be 250 mg and 2 g respectively by the European Food Safety Authority (EFSA, 2009). For a food source to be classed as a ‘source of’ or ‘high in’ *n*-3 PUFA (DHA+EPA), it should contain 40 or 80 mg EPA+DHA per 100 g and per 100 kcal, respectively (EFSA, 2009). Increasing the fatty acid profile of beef in line with current health recommendations would further enhance the nutritional properties of beef, and therefore its perceived food value.

Rearing cattle on grass-based diets, which are rich in 18:3*n*-3, improves the muscle deposition of *n*-3 fatty acids and 18:1 *cis*-9, and lowers the SFA and 16:0 deposition, relative to *n*-6 fatty acid rich concentrate-based diets (Gatellier, Mercier, Juin, & Renerre, 2005; Nuernberg, et al., 2005; Shingfield, et al., 2013; Warren, Scollan, Enser, et al., 2008; Shingfield, Bonnet, & Scollan, 2013), however, not all cattle can be reared or finished on pasture. Pasture feeding lucerne to cattle, also increased the dietary intake of 18:3*n*-3; which increased muscle *n*-3 PUFA content without altering the amount of muscle *n*-6 PUFA, thereby improving the *n*-6:*n*-3 (Blanco, et al., 2010). Macerating the leafy material of Lucerne, centrifuging and heating the extracted juice, produced a protein-xanthophyll-rich (PX) that can be used as a dietary supplement for humans and animals (Grela & Pietrzak, 2014; Rechulicz, Ognik, & Grela, 2014). This low fibre product has 50% of its fatty acid

content as 18:3 *n*-3 (Grela & Pietrzak, 2014), which may have some protection from rumen biohydrogenation. The PX product has been shown to increase the *n*-3 fatty acid content of bovine milk (Dang Van et al., 2011).

There are challenges to enhancing the amount of PUFA in beef. Dietary PUFA are extensively biohydrogenated in the rumen, in which unsaturated fatty acids are isomerised and hydrogenated by ruminal microbes to form SFA, in particular 18:0 (Buccioni, Decandia, Minieri, Molle, & Cabiddu, 2012; Chikunya, et al., 2004; Jenkins & Bridges, 2007). Feeding protected sources of dietary PUFA can help ameliorate the effects of biohydrogenation (Gulati, Garg, & Scott, 2005; Jenkins & Bridges, 2007) and previous studies have demonstrated that PX may provide a degree of protection in the rumen (Garcia-Galicia, Richardson, Ball, Coulmier, & Scollan, 2009; Kim, et al., 2009). Additionally, the source of the dietary PUFA can have important effects on the flavour and storage life of the resulting beef products. Cattle diets containing fish oil were highly effective at increasing the long chain *n*-3 fatty acids, however the meat was more susceptible to lipid and colour oxidation and had impaired flavour characteristics (Vatansever, et al., 2000). The reduced oxidative stability of meat that is associated with increased PUFA incorporation in meat can be countered by increasing the intake of vitamin E, either by feeding a diet containing naturally occurring vitamin E, such as grass, or through the addition of dl-alpha tocopherol acetate. Addition of PX to concentrates increased muscle PUFA and concomitantly decreased oxidative stability of muscle relative to a control concentrate and this caused a decline in shelf life (Kim et al 2009 and Garcia-Galicia et al 2009). In these same studies, feeding grass silage increased both vitamin E and PUFA, thus protecting oxidative stability and shelf life of muscle, therefore the authors concluded that additional vitamin E is required where PX is added to concentrate.

The present study explored the potential to enhance the *n*-3 PUFA muscle profile of cattle through dietary inclusion of PX. Three main hypotheses were tested: 1) In the absence of PX, confirmed that feeding a grass silage based diet (*n*-3 rich) elevated the *n*-3 PUFA profile of beef compared to feeding a concentrate-based diet (*n*-6 rich). 2) Explored the potential of PX (75 or 150 g PX/kg dry matter intake, DMI) to further enhance the muscle PUFA profile from grass silage fed animals, in order to produce beef that could be deemed a 'source of' key

PUFA in line with the EFSA guidelines. 3) Compared the fatty acid profile of beef from animals fed PX extract plus concentrates to that of animals fed a diet of grass silage $\pm$ PX. As concentrate based diets have low background amounts of vitamin E, cattle fed concentrates plus PX were also fed vitamin E to stabilise the effects of increased PUFA. The concentrate plus PX ration was targeted to match the higher inclusion rate of PX on forage (150g PX/kg DMI), to observe whether the shift in muscle PUFA by PX-concentrate fed animals would match that of grass silage or even be sufficient to also be considered a ‘source of’ key PUFA.

2. Materials and methods

2.1. Animals and diets

Forty Belgian Blue cross steers (initial age 20-25 weeks) were housed at the Gogerddan Beef Research Unit, Aberystwyth University and maintained on grass silage in winter and on pasture during spring/summer respectively. At the end of this period, animals were housed in a well-ventilated barn, bedded on wood shavings with *ad libitum* access to water and mineral blocks, and fed individually using electronic weigh scale “Hoko feeders” (Insentec, Marknesse, The Netherlands) for the remainder of the study. A Hoko feeder bin was allocated to each animal to record individual feed intake using the computerised system. Animals were acclimatised to the Hoko feeders by feeding *ad libitum* grass silage over a 4-week period, prior to being weighed on two consecutive days and allocated to an experimental group, balanced for animal age (days -35) and live weight using GenStat® (14th Edition) COVDESIGN.

Animals (n = 8/diet) were assigned to one of five diets: 1) *ad libitum* grass silage (GS), 2) *restricted* grass silage plus 75 g PX crumb/kg silage DMI (GS-LPX), 3) *restricted* grass silage plus 150 g PX crumb/kg silage DMI (GS-HPX), 4) *restricted* control concentrate plus barley straw (S-CC), or 5) *restricted* PX-concentrate, containing 25% PX (on a DM basis) and 2000 IU vitamin E/kg PX-concentrate plus barley straw (S-PXC). The PX inclusion on diet 5 was expected to deliver to ~150 g PX/total dietary DMI assuming cattle consumed straw and concentrates at a ratio of 40:60 on a DMI basis. Straw was available *ad libitum* to concentrate-fed animals. Animals fed on grass silage (diets 2 and 3) and concentrates (4 and 5) were restricted to help achieve similar live weight gain of animals fed GS (diet 1), by

monitoring the live weight gain of the GS group and using the predicted energy content of the grass silage and concentrates to calculate the amount of diet to be fed. Concentrates offered were wholly consumed, and refusals of straw and silage were removed 3 times a week. The formulation of the concentrates are shown in Table 1. The PX feed component was produced by Désialis (Chalons en Champagne, France) from the liquid fraction of fresh lucerne (*Medicago sativa* L.) that had been extracted, heat-treated and dried and fed as a crumb, or incorporated and pelleted into a concentrate feed (Grela & Pietrzak, 2014). Concentrate feeds were prepared by Wynnstay Feeds (Powys, Wales) on a 3-4 monthly basis.

Experimental diets were introduced gradually over a 5-week period. The straw was chopped to a uniform length (8-12 cm) using a mixer wagon and fed using the Hoko feeders. The amount of PX crumb to be fed to the GS-LPX and GS-HPX animals was calculated for each individual every 7 days, based upon the DMI of silage from the previous week. The DM of feeds was assessed three times per week for silage and straw, and weekly for the concentrates, using a forced-air oven at 105°C. Feed samples were bulked over 4-week periods through-out the study for analysis of chemical composition, using the method described by Dewhurst, et al. (1999), or for fatty acid composition as described by (Scollan, et al., 2001). Vitamin E analysis of feed was performed using the method described by Pocklington and Dieffenbacher (1988). The chemical composition of experimental feeds is shown in Table 2.

2.2. Slaughter and sampling of animals

Animals were weighed every 14 d at the same time of day and the condition score assessed, through-out the study. Selection for slaughter was based upon the condition score and appearance of each animal, assessed by a single individual expert in such assessment, to target a carcass fat class of 3L (based upon the EUROP grid for classifying carcasses). They were transported to the University of Bristol EU-licenced abattoir the day before slaughter, across a total of 7 slaughter dates. Slaughter was achieved using a captive bolt gun and severing the carotid arteries. Following slaughter, carcasses were exposed to low voltage electrical stimulation (90 volts for 45 s) and chilled overnight in air at 1°C and 0.5m. sec⁻¹. The carcasses were classified for fatness and conformation, using the EUROP system as

described by Kempster, Cook, & Grantley-Smith (1986), by the same individual. The pH of the *M. longissimus* was measured at 3 and 48 h after slaughter using a Testo 230 pH probe (Testo limited, Alton, UK) and calibrated with pH 4.01 and 7.00 buffers. The temperature was recorded simultaneously and used to make a temperature compensated pH measurement. After 48 h, the carcass weight (excluding channel fat and kidney knob) was measured; and a 40 cm long section of the *M. longissimus thoracis*, posterior to the 10-11th rib, was removed. Separate 20 mm serial cross sections for each analysis were cut from the loin and dissected free from subcutaneous adipose tissue, prior to being vacuum packed and frozen at -20°C until later analysis for vitamin E content, fatty acid composition and CLA profile. A further 20 mm thick steak was weighed, hung in an inflated plastic bag in a chiller (1°C) for 48 hours and then reweighed as a measure of drip loss. An additional steak was sampled, placed on a polystyrene tray, overwrapped with oxygen permeable film and allowed to bloom for one hour and then the colour measured using a Minolta CR400 (Minolta camera Company, Milton Keynes, UK) with an open cone for measuring through the package surface. Illuminant D65 0/45 standard observer 10°C as per recommendations of expert working group (Cassens et al., 1995). The remaining loin was vacuum packed whole, and conditioned at $0 \pm 1^\circ\text{C}$ for a further 10 d after which four, 20 mm thick steaks were individually packaged in modified atmosphere packaging (MAP, 80% Oxygen:20% Carbon dioxide) and displayed in a chiller under simulated retail display conditions (3°C, for 16h light:8h dark, 700 lux). A 75 mm section was vacuum packed, conditioned a further two days, to a total of 14 days from slaughter, then frozen for subsequent analysis by a trained sensory panel.

2.3. Fatty acid/CLA isomer analysis

The intramuscular fat of the *M. longissimus* was extracted in chloroform:methanol (2:1) using the method described by Folch, Lees, & Sloane Stanley (1957) and separated into neutral lipid and phospholipid fractions using silicic acid chromatography for analysis of fatty acids in each fraction according to the method described by Demirel *et al.* (2004). The neutral and phospholipid fractions were separately analysed for fatty acid composition using gas liquid chromatograph (GC) with a 50 m column, using standard procedures as described by Scollan, et al. (2001) using diazomethane as the methylating agent. Briefly, the samples were diluted in n-hexane (20 mg lipid/ml), using 1 µl injection split 60:1. The gas carrier was helium (0.4ml/min) run on a temperature program of 165°C, held for 10 min; then increased at 5°C/

min to 200°C, held for 30 min; then increased at 5°C/min to 220°C and held for 1 min. Fatty acid composition was quantified using heneicosanoic acid methyl ester as an internal standard and is reported as mg per 100 g wet tissue. The fatty acids reported in this study represent >90% of the total fatty acids present. The fatty acid 16:1*cis* refers to both the *n*-7 and *n*-9 isomers and contaminating branched 17-carbon fatty acids. The 18:1*trans* isomers are not individually identifiable using this method, and are referred to as a single value. The 18:1 isomers listed in this study may also have some minor cross-contamination as a consequence of the wide range of these isomers in ruminant tissues (Hay & Morrison, 1973).

The CLA isomers in the extracted total lipids of *M. longissimus* were analysed using silver ion high pressure liquid chromatography (HPLC) using an HPLC system (LC 10A, Shimadzu, Kyoto, Japan) equipped with a pump (LC-10AD VP), auto sampler (SIL-10AF), 50 µL injection loop, a photodiode array detector (SPD-M 10Avp, Shimadzu, Kyoto, Japan) operated at 233 nm, and a Shimadzu CLASS-VP software system (Version 6.12 SP4), as described in detail by Shen, Dannenberger, Nuernberg, Nuernberg, & Zhao (2011). Briefly, four ChromSpher 5 Lipids silver ion-impregnated columns (4.6 mm i.d. × 250 mm stainless steel; 5 µm particle size; Agilent Technologies, Santa Clara, USA) were used in series. Freshly prepared mobile phase, consisting of 0.1% acetonitrile, 0.5% diethyl ether in *n*-hexane, was pumped at a flow rate of 1.0 mL/min. The injection rate of CLA was between 15-30 µL, depending on the total amount of CLA. The CLA isomers were quantified by the measurement of the integrated area under the 233 nm peaks attributed to conjugated dienes. The classification of CLA isomers was achieved by reference to the retention time of the individual isomers of CLA methyl esters (*cis*-9,*trans*-11 CLA, *trans*-9,*trans*-11 CLA, *trans*-10,*cis*-12 CLA, *cis*-9,*trans*-11 CLA, *cis*-9,*cis*-11 CLA and *cis*-11,*trans*-13 CLA). Other isomers were identified by their known elution order, as determined previously by Fritsche, et al.(2000). The external calibration plots of the standard solutions were adapted to different concentration levels of individual CLA isomers in the lipid extracts, as described previously by Dannenberger, et al. (2005). The concentrations of CLA isomers are expressed as mg/100 g fresh muscle. The detection limit of CLA isomers was calculated from the fivefold signal/noise ratio (<0.02 mg/100 g fresh tissue).

2.4. Meat colour and chemical analysis

The colour of duplicate steaks packed in MA was measured daily at 3 positions of the meat surface, through the film lid of the pack using a Minolta Chromameter (Minolta Camera Company, Milton Keynes, UK). A white tile covered by the film lid of MAP was used to standardise the chromameter. Colour shelf life was measured daily until a chroma of ≤ 18 was obtained, which is a critical threshold at which consumers can detect discolouration (Hood and Riordan, 1973; MacDougall, 1982). Colour saturation (chroma) and hue were calculated as

$$\text{Chroma} = [(a^*)^2 + (b^*)^2]^{0.5} \text{ and Hue} = \arctan b^*/a^*$$

The remaining two MA packed steaks were removed from packaging on day 10 of display and trimmed of visible fat, for measurement of thiobarbituric acid reacting substances (TBARS) as described by Tarladgis, Watts, Younathan, & Dugan (1960), with the exception that a Buchi 321 distillation unit was used in the present study. Absorbance was read at 532 nm using an ultraviolet/visible spectrophotometer.

The vitamin E content of meat was measured according to the methodology described by Arnold, Scheller, Arp, Williams, & Schaefer (1993). Rac-5,7-dimethyl-tocol solution was used as internal standard, and 4% dioxane in hexane was used as the mobile phase for HPLC.

2.5. Sensory assessment

The sensory analysis was performed for each animal by a 10-person trained professional taste panel, using the same people for the duration of the study (British Standards Institution, 1993), across 8 sessions. The loin was thawed over night at 4°C and cut into 20 mm thick steaks. Steaks were grilled to an internal temperature of 74°C, measured using a thermocouple probe (Testo limited, Alton, UK). Following cooking, all fat and connective tissue was removed and the steak was cut into 2 cm³ cubes. The samples were placed into pre-labelled foils and placed in a heated incubator at 65°C. Assessors tasted the samples in an order based on the designs outlined by MacFie *et al.* (1989) for balancing carryover effects between samples.

All sensory assessments were completed under red light in a purpose-built sensory suite where each tasting booth was equipped with computer terminals linked to a fileserver running

a sensory software programme (Fizz v 2.20h, Biosystemes, Couternon, France). Panellist assessed one sample from each diet per session, four sessions in a morning. Steaks were scored against 0 - 100 mm unstructured intensity line scales for a consensually agreed texture profile, where 0 = nil and 100 = extreme, and 8-point category scales for tenderness (1 = extremely tough to 8 = extremely tender), juiciness (1 = extremely dry to 8 = extremely juicy), beefy flavour and abnormal beef flavour intensities (1 = extremely weak to 8 = extremely strong). The hedonic scale served as an indication of preference by the panel, but it cannot be used to infer consumer acceptance since the results are based on 10 assessors who can no longer be considered as typical consumers because of the training they have received in meat assessment.

2.6. Statistics

Data are expressed as arithmetic mean, unless otherwise stated. Where stated, Log_{10} transformation was used, as necessary to satisfy the assumptions of the statistical analysis; in these instances, the geometric mean of back transformed data is shown. All statistical analysis was performed using GenStat® software, 14th Edition (Payne, Murray, Harding, Baird, & Soutar (2011). Animal, feed, meat chemical composition and sensory characteristic data were analysed by ANOVA with diet as the main effect. For sensory characteristics, samples were blocked by diet and assessor and sensory session were treated as fixed effects without interactions with the other fixed effects. Multiple comparisons were performed using Tukey's *post hoc* test. The standard error of the difference (sed) from the analysis is shown, and a *P* value of < 0.05 was taken as significant for all statistical analysis.

3. Results

3.1. Animal Feed and Performance

The total feed intake of cattle was greater for animals receiving a concentrate-based diet (S-CC and S-PXC) compared to animals receiving a forage-based diet ($P < 0.05$, Table 3). Consumption of PX differed between treatments and was in the order of S-PXC>GS-HPX>GS-LPX ($P < 0.05$, Table 3). The live weight gain, age at slaughter, carcass conformation, drip loss and fatness and half carcass cold weight were similar across all diets (Table 3). Muscle pH of all carcasses at 48 h was <6 . An overall treatment effect on pH was observed, however Tukey's post hoc could not distinguish a difference between the treatment groups (Table 3).

Analysis of the dietary fatty acid intake by cattle demonstrated that, as expected, there were significant differences between diets (Table 4). Inclusion of the PX extract to either concentrate or grass silage increased the total dietary intake of 18:3 n -3 ($P < 0.05$, Table 4). However the forage-based diets (GS, GS-LPX and GS-HPX) still provided the greatest total dietary intake of 18:3 n -3, compared to the animals receiving either S-CC or S-PXC diets ($P < 0.05$; Table 4). In contrast, the reverse was true for the SFA and 18:2 n -6, with the highest intakes associated with the S-CC and S-PXC diets ($P < 0.05$; Table 4). Vitamin E content of feeds was 28.9, 1961.2, 39.2 and 364.1 mg/kg for the control concentrate, the PX-concentrate, grass silage and PX crumb, respectively.

3.2 Fatty Acid Profile of the *M. longissimus*

A summary of the total lipid fatty acid profile of the *M. longissimus* is shown in Table 5. The total lipid, total neutral lipid and total phospholipid content of muscle was similar across treatments, as was the concentration and proportion of SFA (Table 5). The proportion of PUFA in muscle was greatest for the S-PXC treatment compared to all other treatments ($P < 0.05$, Table 5). The PUFA concentration of total lipid was lower for GS steaks than for S-CC steaks ($P < 0.05$, Table 5). Addition of PX increased the concentration of PUFA in the total lipid of steaks from the S-PXC treatment compared to S-CC, and for steaks from the GS-HPX diet compared to GS ($P < 0.05$, Table 5). Further examination of the PUFA showed that

the muscle concentration and proportion of *n*-6 fatty acids was greatest for concentrate-based diets, compared to the forage-based diets, whereas forage-based diets were associated with greater concentrations of muscle *n*-3 fatty acids compared to the S-CC diet ($P < 0.05$, Table 5). Compared to the respective control (i.e. GS vs. GS-HPX, or S-CC vs. S-PXC), addition of PX into a concentrate or forage diet did not significantly increase the muscle content or proportion of sum *n*-6 PUFA, however it did elevate the total *n*-3 PUFA in the muscle, with subsequent improvement in the *n*-6:*n*-3 PUFA ratio ($P < 0.05$, Table 5). The P:S (PUFA:SFA) ratio was elevated for muscle from cattle fed the S-PXC diet, compared to all other treatments ($P < 0.05$, Table 5). However, addition of PX to either concentrate or grass silage tended to elevate the amount of EPA plus DHA in muscle, and this trend was significant when added at the rate of 150 g PX/kg silage compared to grass silage alone ($P < 0.05$, Table 5).

Finally, the MUFA content of muscle was unaffected by diet, but there was a dietary effect on the proportion of MUFA, with the S-PXC diet resulting in the lowest proportion of MUFA compared to all other diets ($P < 0.05$, Table 5).

Assessment of the fractionated lipids demonstrated that within the neutral lipid fraction, diet only significantly affected the 18:2*n*-6 and 18:3*n*-3 concentration of muscle ($P < 0.05$, Table 6). The muscle concentration of 18:2*n*-6 was greater for concentrate-based diets compared to forage-based diets ($P < 0.05$, Table 6). The highest neutral lipid concentrations of 18:3*n*-3 were observed for the S-PXC and GS-HPX diets, whereas the S-CC and GS diets resulted in the lowest concentrations of this fatty acid in the neutral lipid ($P < 0.05$, Table 6). Steaks from animals fed GS-LPX had similar 18:3*n*-3 concentration in the neutral lipid, as steaks from cattle fed GS, S-PXC and GS-HPX (Table 6).

The fatty acid content of the phospholipid fraction of the *M. longissimus* showed greater susceptibility to dietary effects (Table 6). Whilst the reported SFA (14:0, 16:0 and 18:0) were unaffected by diet; the amount of MUFA fatty acids 16:1, 18:1 *trans* and 18:1 *cis*-9 in the phospholipid varied between treatments, where GS resulted in higher 16:1 and 18:1 *cis*-9 but lower 18:1 *trans*, compared to S-CC ($P < 0.05$, Table 6). Addition of PX caused 16:1 and

18:1 *cis*-9 to decline compared to S-CC, whereas in the forage diets the GS-LPX diet also had lower 16:1 but the GS-HPX diet increased 18:1 *trans* compared to the GS diet ($P < 0.05$, Table 6). The amount of 18:2 n -6 in the phospholipid fraction was greatest in muscle from cattle fed concentrate-based diets (S-CC and S-PXC) than the forage-based diets (GS, GS-LPX, GS-HPX) ($P < 0.05$, Table 6). Addition of PX to either concentrate- or forage-based diets enhanced the amount of 18:3 n -3 in the muscle phospholipid, relative to the respective control diets. The amount of 18:3 n -3 was similar between cattle fed either S-PXC or GS within the phospholipid fraction ($P < 0.05$, Table 6). The amounts of long chain n -3 and n -6 fatty acids in the phospholipid fraction were increased and decreased respectively, by addition of PX to the concentrate diet (S-CC vs. S-PXC, $P < 0.05$). However, the forage-based diets (plus or minus additional PX) still delivered greater long chain n -3 muscle phospholipid content compared to concentrate diets, whereas the reverse was true for the long chain n -6 PUFA ($P < 0.05$, Table 6). When added to the grass silage, PX only enhanced 20:5 n -3 (EPA) relative to feeding grass silage alone (GS vs. GS-HPX, $P < 0.05$, Table 6).

Thirteen CLA isomers were identified within the total lipid fraction of the *M. longissimus*, of which only CLA *trans*-11, *trans*-13 and CLA *trans*-11, *cis*-13 were affected by dietary treatment (Table 7). The amount of CLA *trans*-11, *trans*-13 detected in muscle from cattle fed S-CC was lower than that identified in muscle from cattle fed the GS-LPX diet ($P < 0.05$, Table 7). The CLA isomer, CLA *trans*-11, *cis*-13, showed a similar trend, being lowest in the S-CC cattle compared to both GS-LPX and GS-HPX cattle (Table 7, $P < 0.05$).

3.3 Shelf Life and Sensory Characteristics of the *M. longissimus*

The colour chroma of all steaks declined during simulated retail storage with values declining below 18 at 15 to 20 days (Figure 1). The S-PXC diet produced steaks with 2 days longer colour shelf life than those from the S-CC, GS or GS-HPX diets. Those from the GS-LPX diet were intermediate (Table 8). After 10 days of storage, steaks from cattle fed S-PXC were the least susceptible to lipid oxidation, and had greatest muscle vitamin E content compared to all other treatments ($P < 0.05$, Table 8). The GS diet was associated with lower TBARS compared to S-CC, despite both treatments resulting in similar muscle vitamin E content ($P < 0.05$, Table 8). Addition of PX to the forage-based diets increased the susceptibility to lipid oxidation compared to GS alone ($P < 0.05$, Table 8).

The loin steaks from cattle fed the GS diet were tougher than steaks from cattle fed the other diets ($P < 0.05$, Table 9). The GS steaks also scored highly for the descriptors ‘greasy’ and ‘bloody’ compared to steaks from cattle fed the S-PXC diet ($P < 0.05$, Table 9). However, the overall liking of steaks was similar across all diets (Table 9).

4. Discussion

The FAO and WHO recommend that the human diet needs to provide appropriate amounts of 18:3 n -3 and 18:2 n -6, which are essential fatty acids, but also the long chain n -3 fatty acids, EPA and DHA, to gain the physiological benefits of these fatty acids (FAO, 2010; World Health Organisation, 2003). Increasing the intramuscular fat content of PUFA and n -3 fatty acids in beef products is in line with this recommendation. Concentrate feeding of beef cattle is associated with an increase in SFA and n -6 fatty acids in beef products; whereas grass-based diets enhance the PUFA and n -3 fatty acids in meat (Marmer, Maxwell, & Williams, 1984; Scollan, et al., 2014). Through the addition of an 18:3 n -3 rich extract from lucerne (PX) to the cattle diets, the present study attempted to 1) further enhance the n -3 PUFA muscle profile of forage fed animals, resulting in beef that could be deemed a 'source of' key PUFA; 2) alter the fatty acid profile of beef of concentrate fed animals to also achieve this aim using a concentrate-based ration, or at least achieve similar n -3 PUFA composition to that of forage fed animals.

4.1 Animal Performance

Steers achieved similar live weight gains, and accordingly were slaughtered at similar ages across diets, despite differences in the total feed intake of concentrate versus forage fed animals. Additionally, half carcass weight, carcass conformation and fatness were similar across all diets therefore differences in the total fatty acid profile of muscle can be attributed to dietary effects, rather than any confounding effects of animal body composition on total fatty acid profile of muscle (Enser, et al., 1998; Marmer, et al., 1984). Differences in pH were observed as an overall treatment effect, but not in the *post hoc* analysis, suggesting a marginal difference between treatments. However as the muscle pH was <6 in all cases it is unlikely to be of practical importance. Increased carcass fatness is associated with a predominance of SFA in total lipid, which reflects an increase in intramuscular neutral lipid (NL) as fat deposition progresses. In contrast, the phospholipid fraction (PL) is more constant and less susceptible to shifts in body composition. The PL fraction, which forms muscle cell membranes, is more unsaturated due to the functional presence of long chain PUFA (Scollan, et al., 2001; Wood, et al., 2008). As the PL fraction is more constant, the proportion of PUFA decreases as total lipid increases and fatness increase (Enser, et al., 1998; Marmer, et al., 1984; Warren, Scollan, Enser, et al., 2008). In the present study, the carcass fatness and the amount of total lipid in muscle were similar across diets, so the use of proportions is valid and useful to summarise the dietary effects on fatty acid profile of muscle. However

concentrations of fatty acids in meat are more meaningful when attributing nutritional value (Enser, et al., 1998; Marmer, et al., 1984), therefore individual fatty acids in each lipid fraction were shown as mg/100g fresh muscle.

4.2 Fatty Acid Profile of Longissimus Muscle

The addition of PX to the concentrate diet resulted in a decrease in the proportion of MUFA and an increase in the proportion of PUFA in the *M. longissimus* compared to the other diets. The most abundant MUFA in beef is oleic acid (18:1*cis*-9) (Marmer, et al., 1984), and in this study cattle fed the S-PXC diet showed a decline 18:1*cis*-9 in the NL and PL fractions which was non-significant and significant, respectively. Interestingly, the ratio of 18:0/18:1 (as a proxy of stearoyl-CoA desaturase (SCD)) was higher for the S-PXC versus S-CC 0.48 and 0.42, respectively; $P < 0.003$), suggesting a lower conversion of 18:0 to 18:1 in muscle. Cattle fed concentrates (with no additional lipid) compared to forage are typically associated with greater accumulation of MUFA, in particular, 18:1*cis*-9, (Daley, Abbott, Doyle, Nader, & Larson, 2010). In humans, dietary intake of MUFA has been associated with health benefits which include preventing cardiovascular disease, lowering cholesterol and reduced risk of stroke (Daley, Abbott, Doyle, Nader, & Larson, 2010). In the present study, no difference between muscle content of 18:1*cis*-9 between basal concentrate (S-CC) versus the basal forage (GS) diet was found, despite differences in the intake of 18:1*cis*-9 between diets. In agreement, others have identified that in ruminants, *de novo* synthesis of 18:1*cis*-9 is of greater importance than the amount consumed (Daniel, Wynn, Salter, & Buttery, 2004). Concentrate diets have been found to increase the expression of SCD relative to forage-based diets (Daniel, et al., 2004; Hiller, 2014), where SCD desaturates stearic acid (18:0) to form 18:1*cis*-9 (see review, Alvarenga, Chen, Furusho-Garcia, Perez, & Hopkins, 2015). In the present study, the ratio of 18:0/18:1 in muscle was similar for steaks from cattle fed either S-CC or GS, however the ratio of 18:1 *trans*-11/CLA *cis*-9, *trans*-11 was significantly lower for S-CC steaks versus GS steaks (0.19 vs 0.23 for S-CC and GS respectively, $P = 0.011$), which may indicate greater conversion of 18:1 *trans*-11 to CLA *cis*-9, *trans*-11, possibly via higher SCD activity in the muscle of S-CC animals.

The S-PXC diet resulted in a greater proportion of sum *n*-3 PUFA and sum *n*-6 PUFA in the intramuscular fat of *M. longissimus* than the unsupplemented concentrate diet (S-CC), and reflects the higher proportion of intramuscular PUFA in cattle fed the S-PXC diet.

Examination of the individual fatty acids in the PL and NL fractions showed a significant

increase in intramuscular 18:3 n -3 in both the NL and PL. Linoleic acid (18:2 n -6) and 18:3 n -3 cannot be synthesised *de novo* in body tissue, thus the diet is the only source of these fatty acids for incorporation into muscle. However significant losses of dietary PUFA occur during ruminal lipolysis and biohydrogenation (85-100% of 18:3 n -3 and 60-95% for 18:2 n -6) (Doreau & Ferlay, 1994; Jenkins & Bridges, 2007). The PX supplement provides a rich supply of 18:3 n -3, but also 18:2 n -6 (Grela & Pietrzak, 2014), yet in the present study the daily intake of 18:2 n -6 was lower for S-PXC than S-CC (S-PXC: 124.6 g/day vs. S-CC: 158.8 g/day). Thus the greater incorporation of 18:2 n -6 into muscle on the S-PXC muscle, compared to the S-CC diet, suggests that there is a greater efficiency of delivery of this essential fatty acid via the PX supplement when comparing the concentrate rations.

Steaks from the S-PXC diet were also associated with greater or equal incorporation of 18:3 n -3 into the intramuscular fat of *M. longissimus* compared to the GS diet, for the neutral and phospholipid respectively. When compared to the GS-HPX, steaks from the S-PXC diet had similar incorporation of 18:3 n -3 in the neutral lipid. All the forage-based diets (GS, GS-LPX and GS-HPX) were associated with greater dietary intake of 18:3 n -3 compared to the S-PXC diet, which is not surprising as the grass silage had higher background amounts of 18:3 n -3 compared to concentrate (Table 2), and as observed in the wider literature (Dewhurst, Scollan, Lee, Ougham, & Humphreys, 2003; Warren, Scollan, Enser, et al., 2008).

The greater efficiency for incorporation of 18:2 n -6 and 18:3 n -3 by PX-based diets may therefore indicate that the PUFA associated with PX have some degree of protection from ruminal biohydrogenation. In support of this theory is the finding that the 18:3 n -3 and 18:2 n -6 in the alfalfa extract underwent less biohydrogenation than the same fatty acids in extruded linseed or extruded rapeseed (*in vitro* biohydrogenation of 18:3 n -3: 67% vs. 92% and 91% for alfalfa extract vs. extruded linseed or extruded rapeseed) (Dang Van et. al, 2011). Losses of dietary PUFA to biohydrogenation can be reduced by feeding protected lipid sources, thus resulting in greater incorporation of these PUFA into muscle (Jenkins & Bridges, 2007). The PX product is not a protected lipid source *per se*, however it is a protein rich extract (533.9 g/kg) from the liquid fraction of lucerne which undergoes a vapour heating process (85-90°C) during the extraction to maximise precipitation of proteins, followed by a low temperature heating process to prevent denaturation of the extracted proteins (Grela & Pietrzak, 2014). It

is possible that there is some degree of protection afforded to the PX lipid by the extracted proteins during the heating process, hence greater delivery of the dietary PUFA into muscle compared to the S-CC diet. Differences may also in part relate to the acknowledged greater degree of biohydrogenation on high forage relative to high cereal-based concentrate rations (Dewhurst, et al., 2003). Additionally, it has been shown that supplemental vitamin E can modify the biohydrogenation products, resulting in increased muscle deposition of *n*-3 fatty acids (Juárez, et al., 2011). Cattle fed on the S-PXC diet also had supplemental vitamin E, which could have contributed to differences in muscle PUFA. However, the data in the present study do indicate that addition of PX to concentrate can facilitate an increase in muscle content of 18:3*n*-3 to match that of beef from cattle fed a grass-based diet, thus fulfilling one of the major objectives of this study.

The rationale behind this objective was underpinned by the consistent finding that grass-based diets elevate the amount of muscle *n*-3 PUFA (18:3*n*-3 and long chain *n*-3 PUFA), reduce or result in similar *n*-6 PUFA content, and therefore improve the *n*-6 to *n*-3 PUFA ratio in beef compared to concentrate feeding (Daley, et al., 2010; Dewhurst, et al., 2003; Marmer, et al., 1984; Warren, Scollan, Enser, et al., 2008). These effects are consistently observed despite the extensive biohydrogenation of PUFA by the rumen (Doreau & Ferlay, 1994). The results of the present study are coherent with this theory for both 18:3*n*-3 and the long chain *n*-3 PUFA. A shift in the long chain *n*-3 fatty acids was apparent in the muscle PL from cattle fed GS, compared to S-CC, with subsequent improvement in EPA+DHA. This supports the proposition that essential fatty acids and functional long chain fatty acids are preferentially conserved in the PL fraction, whereas the NL is more saturated and serves as an energy store (Wood, et al., 2008).

4.3 Effect of Cattle Diet on CLA Isomers

Intake of CLA in the human diet has been attributed to a range of health benefits, including anti-carcinogenic effects, reduction in atherosclerosis, and prevention of onset of diabetes (Belury, 2002). Beef is an important source of CLA in the human diet, and the CLA isomer 18:2 *cis*-9, *trans*-11 (CLA *cis*-9, *trans*-11) was the most abundant CLA isomer observed in the total lipid of *M. longissimus*, consistent with previous reports (FAO, 2010; Mapiye et al. 2013). Both 18:3*n*-3 and 18:2*n*-6 can act as pre-cursors for CLA in the rumen. Formation of CLA *cis*-9, *trans*-11 occurs via desaturation of 18:1*trans*-11 by SCD enzyme, but also by partial ruminal biohydrogenation of 18:2*n*-6 (Daley, et al., 2010; Dewhurst, et al., 2003;

Griinari, et al., 2000). Previous reports have demonstrated that grass feeding can increase CLA in muscle, in part due to favourable effects of ruminal pH on biohydrogenation (Dannenberger, et al., 2004; Dewhurst, et al., 2003; Daley, et al., 2010), however in the present study the amount of CLA *cis*-9, *trans*-11 in the intramuscular fat was unaffected by diet. This may be explained by the fact that fresh grass, as opposed to ensiled grass which was fed in the present study, has a greater effect on CLA *cis*-9, *trans*-11 (Dewhurst, et al., 2003). In addition, in dietary studies with supplements rich in 18:3 n -3, the main CLA isomer the CLA *cis*-9, *trans*-11 concentration tended to be unaffected or slightly decreased by pasture feeding (Scollan et al. 2014). In agreement, expression of the SCD gene was down-regulated by diets rich in n -3 PUFA (Hiller, Herdmann, & Nuernberg, 2011; Waters, Kelly, O'Boyle, Moloney, & Kenny, 2009). Other reports show that concentrate feeding based on supplements rich in 18:2 n -6 resulted in higher accumulation of CLA *cis*-9, *trans*-11 compared to pasture feeding (Nuernberg, et al., 2002). Together these findings reflect the complexity of the effect of ruminal biohydrogenation, and expression of lipogenic genes between different diets.

The finding that CLA *trans*-11, *trans*-13 was increased in the *Longissimus* from cattle fed the GS-LPX diet, and cattle fed the GS-LPX and GS-HPX diets accumulated greater intramuscular CLA *trans*-11, *cis*-13, compared to S-CC, is consistent with results of other studies, which showed that pasture-based diets with/without supplements containing linseed/rapeseed cake or oil led to higher muscle concentrations of trans,trans CLA isomers (mainly CLA *trans*-11, *trans*-13; CLA *trans*-12, *trans*-14) and CLA *trans*-11, *cis*-13 (Dannenberger et al., 2005, Alfaia et al., 2009).

4.4 Nutritional Indices

There was an improvement in the P:S ratio following addition of PX to concentrate, compared to concentrate or grass silage alone. The P:S ratio is useful as the FAO have concluded that there is 'convincing' evidence that replacing SFA with PUFA in the human diet can decrease the risk of coronary heart disease in humans (FAO, 2010). In contrast the addition of PX to concentrate was associated with a decline in the n -6: n -3 PUFA ratio, however, the importance of this ratio has recently been questioned by the FAO, as the amount of n -6 and n -3 fatty acids consumed in the human diet is more important and should be within the guideline amounts (0.5 -2% of energy or 2-3% of energy for 18:2 n -3 and 18:2 n -6 respectively) (FAO, 2010).

A second objective of this study was to further improve delivery of 18:3 n -3 through addition of PX to grass silage, to observe whether beef could be considered a 'source of' key fatty acids, namely 18:3 n -3 and the long chain n -3 fatty acids in order to produce 'healthier' beef products. In order to be considered a 'source of', food must contain 15% of the nutrient reference intake, which for EPA+DHA is 250 mg/day, therefore a food must contain at least 37.5 mg/100 g EPA+DHA (EFSA, 2005; FAO, 2010). Addition of PX to concentrate increased the amount of EPA (20:5 n -3), but not the other n -3 long chain fatty acids, in the PL compared to feeding S-CC. A similar pattern was observed for GS-HPX compared to GS, with the GS-LPX providing intermediate amount of EPA. This is consistent with previous observations that grass feeding appears particularly efficient at elevating DHA (Warren, Scollan, Enser, et al., 2008), but other sources of n -3 fatty acids may be less successful at increasing this long chain fatty acid (Scollan, et al., 2001; Wood, et al., 2008). Despite the significant impact of PX on EPA in muscle, the nutritional index EPA+DHA was only significantly increased for the GS-HPX diet compared to S-CC, S-PXC, GS, but this index was still 50% lower than the recommended amount set out by EFSA in order to be considered a 'source of' long chain n -3 fatty acids.

4.5 Effect of Diet on Meat Stability

The colour of beef is predominantly determined by the oxidative state of the haem pigment, myoglobin (Acebron & Dopico, 2000; Insausti, et al., 1999). The red colour of meat can be attributed to exposure of myoglobin to oxygen to form oxymyoglobin. In contrast to previous findings in which steaks from grass fed cattle have been reported to be darker but also less red, than those fed concentrate (Priolo, Micol, & Agabriel, 2001), the lightness of steaks was unaffected by diet in the present study. However, the GS-HPX diet was less red and less yellow with reduced hue and chroma than the steaks from cattle fed the S-CC diet.

Differences in meat colour have been attributed to differences in ultimate pH and the fat content of carcasses from animals fed different diets (Priolo, Micol, & Agabriel, 2001), but meat colour is a multi-factorial attribute and may be influenced by the amount of exercise pasture-based animals have compared to housed animals (Dunne, Monahan, & Moloney, 2011). In the present study carcass fatness was similar, but muscle pH at 48 h post mortem differed between diets ($P=0.045$), however the Tukey's *post hoc* tests could not distinguish differences between diets. All carcasses achieved a pH <5.8 by 48 h, and therefore were not classified as dark, firm, dry, and would not have affected colour.

Meat colour represents a major factor in consumer purchase decisions, and oxidation of oxymyoglobin results in the formation of metmyoglobin, which imparts a brown colour to meat that consumers find unattractive (Insausti, et al., 1999). Colour of *Longissimus* steaks, in MAP and subject to simulated retail display, was measured until a chroma value <18 was reached. A chroma value of 18 corresponds to the formation of 20% metmyoglobin, the borderline value for consumer rejection during the purchase of fresh beef (Hood and Riordan, 1973; MacDougall, 1982). Colour stability of meat can be optimised where muscle tissue has a vitamin E concentration of 3.0 – 3.5 mg/kg (Liu, Scheller, Arp, Schaefer, & Williams, 1996). In the present study, all steaks had a vitamin E content >3.0 mg/kg muscle, however steaks from cattle fed S-PXC had the greatest concentration (7.7 mg/kg) and steaks from the S-CC diet had the lowest (3.2 mg/kg muscle), with intermediate vitamin E concentrations associated with the grass-based diets. These results reflect the degree of lipid oxidation on day 10, measured using TBARS, which was lowest for the S-PXC steaks; and the longer shelf life of the S-PXC steaks compared to the S-CC, GS and H-PXC steaks. The presence of vitamin E, a potent antioxidant, can reduce the degree of oxidation in meat through interaction with reactive oxygen species (Arnold, et al., 1992; Daley, et al., 2010; Warren, Scollan, Nute, et al., 2008). Increased muscle concentration of vitamin E on the S-PXC diet was expected as the S-PXC concentrate contained 2000 IU vitamin E/kg, which was added as the PX supplement was anticipated to deliver greater amount of PUFA into muscle. Previous studies found that grass silage delivered both increased PUFA and vitamin E in muscle relative to a control concentrate ration, which protected shelf life in the steaks from the grass fed animals (Garcia-Galicia, Richardson, Ball, Coulmier, & Scollan, 2009; Kim, et al., 2009). In these same studies, PX was added to a separate concentrate ration in the absence of additional vitamin E, which increased PUFA but decreased oxidative stability and shelf life of muscle. Steaks from animals fed concentrate plus PX had a muscle vitamin E concentration of 2.87 mg/kg muscle; and vitamin E was identified as critical for protecting the oxidative stability of steaks in the presence of a high PUFA diet (Kim, et al., 2009). Other studies have also demonstrated that grass-based diets typically increase the vitamin E of muscle compared to concentrate based diets (Daley, Abbott, Doyle, Nader, & Larson, 2010; Warren, et al., 2008). Accordingly, vitamin E was added to the PX concentrate ration in the present study whereas it was expected that the grass silage would result in a muscle vitamin E concentration of >3 mg/kg muscle, as was observed. The authors propose that the additional vitamin E, not the PX, enhanced the shelf life of steaks from the PXC diet in the present

study. If PX had independent effects on shelf life, it could be expected that this could be observed within the forage diets, where no additional vitamin E was added. However, despite a rise in total PUFA and TBARS on day 10 of storage in steaks from the GS-HPX diet relative to the GS diet, the overall shelf life (days to chroma 18) was similar. Therefore it is concluded that PX plus vitamin E can be added to the concentrate to enhance the PUFA of muscle and the colour shelf life. However, further studies would be required to confirm the previous finding by Kim et al. (2009) that PX can decrease colour shelf life where muscle vitamin E concentration is <3 mg/kg muscle as a consequence of decreased oxidative stability. Additionally, the elevated TBARS and PUFA in steaks from the GS-HPX diet may indicate that there may be a need to also supplement grass silage diets with additional vitamin E where cattle diets seek to further increase muscle PUFA from that typically associated with grass based diets, but further work is required to confirm this.

Addition of PX to the forage-based diets resulted in similar TBARS values as the S-CC diet on day 10 of display. This confirms the finding that that increasing PUFA can increase the oxidative potential of muscle (Mahecha, et al., 2010; Scollan, et al., 2014). These findings indicate that supplementation of diets that are already rich in *n*-3 fatty acids with additional PUFA may require additional supplementation of vitamin E to ensure the stability of the resulting meat. This is particularly relevant for *n*-3 PUFA rich silage-based diets that are supplemented with additional dietary PUFA, as ensiling grass results in losses of PUFA, but also vitamin E (Cuvelier, et al., 2006; Dewhurst, et al., 2003; Lindqvist, Nadeau, & Jensen, 2012). Therefore when supplemental PUFA are added to silage, additional vitamin E may be required.

4.6 Sensory Attributes of Longissimus Steaks

The only sensory attributes that were altered by dietary treatments were texture, and the flavour notes 'greasy' and 'bloody'. Steaks from cattle fed the GS diet were less tender than the other diets. This is in agreement with other studies that found that grass fed cattle produce meat that is tougher, based on consumer tests, than concentrate/grain fed animals (Davis, Cole, Backus, & Melton, 1981; Sitz, Calkins, Feuz, Umberger, & Eskridge, 2005), but in contrast to studies that identified no differences in the tenderness of meat from animals fed concentrates or grass (Duckett, Neel, Lewis, Fontenot, & Clapham, 2013; French, et al., 2001).

Steaks from the GS diet scored significantly more poorly against the S-PXC diet on the terms ‘greasy’, and ‘bloody’. Whilst the term greasy has been associated with variations in overall fatness, animal fatness was controlled across diets in this study. The attribute ‘greasy’ may also be exacerbated by increasing the amount of *n*-3 fatty acids in muscle (Scollan, et al., 2005), which is in contrast with the present study where the S-PXC and GS-HPX diets had greatest amount of total *n*-3 fatty acids in muscle, but the numerically lowest scores for the term ‘greasy’. Forage-based diets have also been associated with changes in steak flavour, for terms including ‘dairy’, ‘grassy’ and ‘livery’, but these results are not consistent between studies, reviewed by Priolo, et al. (2001). Therefore, feeding cattle diets rich in *n*-3 PUFA may impart changes in flavour characteristics of beef from these animals. Although overall liking is hedonic and is only the opinion of these 10 panelists it does give some indication of which attributes may influence an overall assessment. ‘Overall liking’ was not affected by animal diet, a finding that is consistent with other studies (Warren, Scollan, Nute, et al., 2008), that identified an effect of forage diets on particular flavour attributes, but not overall liking. Other reports suggest that perception of flavour is country-specific, with the US consumer preferring the taste of grain fed beef, and the UK consumer preferring the taste of grass fed beef (Priolo, et al., 2001; Sitz, et al., 2005). Therefore, based on the similarity in overall liking of the steaks, moderating the diets fed to cattle had minimal impact upon the eating quality of beef but significant effects on the fatty acid composition.

4.7 Conclusions

Addition of an extract of lucerne to a concentrate diet resulted in similar muscle concentration of 18:3*n*-3 and EPA compared to feeding unsupplemented grass silage. Addition of PX to grass silage also increased the presence of these fatty acids in muscle. However, the other long chain *n*-3 fatty acids (DHA and DPA) were not increased with PX supplementation. None of the diets achieved sufficient concentrations of *n*-3 fatty acids in muscle to be considered a ‘source of’ these fatty acids for human consumption. This is due to the substantial losses of dietary PUFA to biohydrogenation in the rumen. The PX supplement may have a degree of inherent protection from biohydrogenation, but it seems likely that this protection was insufficient to withstand the longer ruminal retention times and greater degree of biohydrogenation typically associated with forage-based diets. This study highlights the challenges associated with increasing the PUFA content of beef, and provides insights into approaches required to achieve higher levels of PUFA in muscle lipids. Ruminal protection of dietary lipids remains a critical control point. Steaks from the S-PXC diet were associated

with a longer colour shelf, as a consequence of increase muscle vitamin E concentrations. Supplementation of *n*-3 PUFA rich grass silage-based diets with additional PUFA may require additional vitamin E supplementation to ensure protection of oxidative effects of high muscle PUFA content. Although some differences were observed in the taste of steaks from different diets, overall liking was similar. Thus PX can successfully be added to concentrate diets to achieve beef with a similar *n*-3 PUFA profile to that of forage fed animals. Studies should continue to develop opportunities to improve the lipid profile of beef, including addressing the challenge of ruminal lipolysis and biohydrogenation of dietary lipids.

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References

- Acebron, L. B., & Dopico, D. C. (2000). The importance of intrinsic and extrinsic cues to expected and experienced quality: an empirical application for beef. *Food Quality and Preference*, 11(3), 229-238.
- Alfaia, C. P.M., Alves, S. P., Martins, S. I. V., Costa, A. S. H., Fontes, C. M. G. A., Lemos, J. P. C., Bessa, R. J. B., & Prates, J. A.M. (2009). Effect of the feeding system on intramuscular fatty acids and conjugated linoleic acid isomers of beef cattle, with emphasis on their nutritional value and discriminatory ability. *Food Chemistry*, 114(3), 939–946.
- Alvarenga, T. I. R. C., Chen, Y., Furusho-Garcia, I. F., Perez, J. R. O., & Hopkins, D. L. (2015). Manipulation of Omega-3 PUFAs in Lamb: Phenotypic and Genotypic Views. *Comprehensive Reviews in Food Science and Food Safety*, 14(3), 189-204.
- Arnold, R. N., Scheller, K. K., Arp, S. C., Williams, S. N., Buege, D. R., & Schaefer, D. M. (1992). Effect of long- or short-term feeding of alpha-tocopheryl acetate to Holstein and crossbred beef steers on performance, carcass characteristics, and beef color stability. *Journal of Animal Science*, 70(10), 3055-3065.
- Arnold, R. N., Scheller, K. K., Arp, S. C., Williams, S. N., & Schaefer, D. M. (1993). Dietary Alpha-Tocopheryl Acetate Enhances Beef Quality in Holstein and Beef Breed Steers. *Journal of Food Science*, 58(1), 28-33.
- Barceló-Coblijn, G., & Murphy, E. J. (2009). Alpha-linolenic acid and its conversion to longer chain n–3 fatty acids: Benefits for human health and a role in maintaining tissue n–3 fatty acid levels. *Progress in Lipid Research*, 48(6), 355-374.
- Belury, M. A. (2002). Dietary conjugated linoleic acid in health: physiological effects and mechanisms of action. *Annual Review of Nutrition*, 22, 505-531.
- Blanco, M., Casasus, I., Ripoll, G., Panea, B., Alberti, P., & Joy, M. (2010). Lucerne grazing compared with concentrate-feeding slightly modifies carcass and meat quality of young bulls. *Meat Science*, 84(3), 545-552.
- Buccioni, A., Decandia, M., Minieri, S., Molle, G., & Cabiddu, A. (2012). Lipid metabolism in the rumen: New insights on lipolysis and biohydrogenation with an emphasis on the role of endogenous plant factors. *Animal Feed Science and Technology*, 174(1-2), 1-25.
- British Standards Institution. 1993. Assessors for sensory analysis. Part 1. Guide to the selection, training and monitoring of selected assessors. BS7667. British Standards Institution, London. BS 7667-1:1993, ISO 8586-1:1993

- Calder, P. C. (2004). n-3 Fatty acids and cardiovascular disease: evidence explained and mechanisms explored. *Clinical Science (London)*, 107(1), 1-11.
- Cassens, R.G., Demeyer, D., Eikelenboom, G., Honikel, K.O., Johansson, G., Nielson, T., Rennerre, M., Richardson, R.I. and Sakata, R. (1995). Recommendation of a reference method for assessment of meat colour. *41st International Congress of Meat Science and Technology*, San Antonio, Texas, Paper C86, pp. 410-411
- Chikunya, S., Demirel, G., Enser, M., Wood, J. D., Wilkinson, R. G., & Sinclair, L. A. (2004). Biohydrogenation of dietary n-3 PUFA and stability of ingested vitamin E in the rumen, and their effects on microbial activity in sheep. *British Journal of Nutrition*, 91(4), 539-550.
- Cuvelier, C., Clinquart, A., Hocquette, J. F., Cabaraux, J. F., Dufrasne, I., Istasse, L., & Hornick, J. L. (2006). Comparison of composition and quality traits of meat from young finishing bulls from Belgian Blue, Limousin and Aberdeen Angus breeds. *Meat Science*, 74(3), 522-531.
- Daley, C. A., Abbott, A., Doyle, P. S., Nader, G. A., & Larson, S. (2010). A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. *Nutrition Journal*, 9(1), 1-12.
- Dang Van, Q.C., Bejarano, L., Mignolet, E., Coulmier, D., Froidmont, E, Larondelle, Y. and Focant, M. 2011. Effectiveness of extruded rapeseed associated with an alfalfa protein concentrate in enhancing the bovine milk fatty acids composition. *Journal of Dairy Science*, 94, 4005-4015.
- Daniel, Z. C. T. R., Wynn, R. J., Salter, A. M., & Buttery, P. J. (2004). Differing effects of forage and concentrate diets on the oleic acid and conjugated linoleic acid content of sheep tissues: The role of stearoyl-CoA desaturase. *Journal of Animal Science*, 82(3), 747-758.
- Dannenberger, D., Nuernberg, G., Scollan, N., Schabbel, W., Steinhart, H., Ender, K., & Nuernberg, K. (2004). Effect of diet on the deposition of n-3 fatty acids, conjugated linoleic and C18:1trans fatty acid isomers in muscle lipids of German Holstein bulls. *Journal of Agricultural and Food Chemistry*, 52(21), 6607-6615.
- Dannenberger, D., Nuernberg, K., Nuernberg, G., Scollan, N., Steinhart, H., & Ender, K. (2005). Effect of pasture vs. concentrate diet on CLA isomer distribution in different tissue lipids of beef cattle. *Lipids*, 40(6), 589-598.

- Davis, G. W., Cole, A. B., Backus, W. R., & Melton, S. L. (1981). Effect of Electrical Stimulation on Carcass Quality and Meat Palatability of Beef from Forage- and Grain-Finished Steers. *Journal of Animal Science*, 53(3), 651-657.
- Demirel, G., Wachira, A. M., Sinclair, L. A., Wilkinson, R. G., Wood, J. D., & Enser, M. (2004). Effects of dietary n-3 polyunsaturated fatty acids, breed and dietary vitamin E on the fatty acids of lamb muscle, liver and adipose tissue. *British Journal of Nutrition*, 91(4), 551-565.
- Dewhurst, R. J., Aston, K., Fisher, W. J., Evans, R. T., Dhanoa, M. S., & McAllan, A. B. (1999). Comparison of energy and protein sources offered at low levels in grass-silage-based diets for dairy cows. *Animal Science*, 68, 789-799.
- Dewhurst, R. J., Scollan, N. D., Lee, M. R., Ougham, H. J., & Humphreys, M. O. (2003). Forage breeding and management to increase the beneficial fatty acid content of ruminant products. *Proceedings of the Nutrition Society*, 62(2), 329-336.
- Doreau, M., & Ferlay, A. (1994). Digestion and Utilization of Fatty-Acids by Ruminants. *Animal Feed Science and Technology*, 45(3-4), 379-396.
- Duckett, S. K., Neel, J. P. S., Lewis, R. M., Fontenot, J. P., & Clapham, W. M. (2013). Effects of forage species or concentrate finishing on animal performance, carcass and meat quality¹². *Journal of Animal Science*, 91(3), 1454-1467.
- Dunne, P. G., Monahan, F. J., & Moloney, A. P. (2011). Current perspectives on the darker beef often reported from extensively-managed cattle: Does physical activity play a significant role? *Livestock Science*, 142(1-3), 1-22.
- Enser, M., Hallett, K. G., Hewett, B., Fursey, G. A. J., Wood, J. D., & Harrington, G. (1998). Fatty acid content and composition of UK beef and lamb muscle in relation to production system and implications for human nutrition. *Meat Science*, 49(3), 329-341.
- EFSA (European Food Safety Authority) (2005). Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to nutrition claims concerning omega-3 fatty acids, monounsaturated fat, polyunsaturated fat and unsaturated fat. *The EFSA Journal*, 253, 1-29.
- EFSA (European Food Safety Authority) (2009). Scientific opinion: Labelling reference intake values for n-3 and n-6 polyunsaturated fatty acids. *The EFSA Journal*, 1176, 1-11.

- Folch, J., Lees, M., & Sloane Stanley, G. H. S. (1957). A Simple Method for the Isolation and Purification of Total Lipides from Animal Tissues. *Journal of Biological Chemistry*, 226(1), 497-509.
- Food and Agricultural Organization of the United Nations (FAO) (2010). Fats and fatty acids in human nutrition. Report of an expert consultation. In FAO (Ed.), *FAO Food and Nutrition Paper*. Rome.
- French, P., O'Riordan, E. G., Monahan, F. J., Caffrey, P. J., Mooney, M. T., Troy, D. J., & Moloney, A. P. (2001). The eating quality of meat of steers fed grass and/or concentrates. *Meat Science*, 57(4), 379-386.
- Fritsche, J., Fritsche, S., Solomon, M. B., Mossoba, M. M., Yurawecz, M. P., Morehouse, K., & Ku, Y. (2000). Quantitative determination of conjugated linoleic acid isomers in beef fat. *European Journal of Lipid Science and Technology*, 102(11), 667-672.
- Garcia-Galicia, I., Richardson, I., Ball, R., Coulmier, K., & Scollan, N. D. (2009). The effect of alpha-tocopherol concentration and antioxidant enzyme activities on the shelf life stability of beef from different feeding systems. In *Advances in Animal Biosciences*. Southport, UK: British Society of Animal Science.
- Gatellier, P., Mercier, Y., Juin, H., & Renerre, M. (2005). Effect of finishing mode (pasture- or mixed-diet) on lipid composition, colour stability and lipid oxidation in meat from Charolais cattle. *Meat Science*, 69(1), 175-186.
- Grela, E. R., & Pietrzak, K. (2014). Production technology, chemical composition and use of alfalfa protein-xanthophyll concentrate as dietary supplement. *Food Processing and Technology*, 5(10), 5-10.
- Griinari, J. M., Corl, B. A., Lacy, S. H., Chouinard, P. Y., Nurmela, K. V. V., & Bauman, D. E. (2000). Conjugated Linoleic Acid Is Synthesized Endogenously in Lactating Dairy Cows by $\Delta 9$ -Desaturase. *The Journal of Nutrition*, 130(9), 2285-2291.
- Gulati, S. K., Garg, M. R., & Scott, T. W. (2005). Rumen protected protein and fat produced from oilseeds and/or meals by formaldehyde treatment; their role in ruminant production and product quality: a review. *Australian Journal of Experimental Agriculture*, 45(10), 1189-1203.
- Hay, J. D., & Morrison, W. R. (1973). Positional Isomers of Cis and Trans Monoenoic Fatty-Acids from Ox (Steer) Perinephric Fat. *Lipids*, 8(2), 94-95.
- Hiller, B. (2014). Recent developments in lipid metabolism in ruminants - the role of fat in maintaining animal health and performance. *Animal Production Science*, 54(10), 1549-1560.

- Hiller, B., Herdmann, A., & Nuernberg, K. (2011). Dietary n-3 Fatty Acids Significantly Suppress Lipogenesis in Bovine Muscle and Adipose Tissue: A Functional Genomics Approach. *Lipids*, 46(7), 557-567.
- Hood, D.E. and Riordan, E.B. (1973). Discoloration in pre-packaged beef: Measurements by reflectance spectrophotometry and shopper discrimination. *Journal of Food Technology*, 8: 333-343.
- Insausti, K., Beriain, M. J., Purroy, A., Alberti, P., Lizaso, L., & Hernandez, B. (1999). Colour stability of beef from different Spanish native cattle breeds stored under vacuum and modified atmosphere. *Meat Science*, 53(4), 241-249.
- Jenkins, T. C., & Bridges, W. C. (2007). Protection of fatty acids against ruminal biohydrogenation in cattle. *European Journal of Lipid Science and Technology*, 109(8), 778-789.
- Juárez, M., Dugan, M. E. R., Aalhus, J. L., Aldai, N., Basarab, J. A., Baron, V. S., & McAllister, T. A. (2011). Effects of vitamin E and flaxseed on rumen-derived fatty acid intermediates in beef intramuscular fat. *Meat Science*, 88(3), 434-440.
- Kempster, A. J., Cook, G. L., & Grantley-Smith, M. (1986). National Estimates of the Body-Composition of British Cattle, Sheep and Pigs with Special Reference to Trends in Fatness - a Review. *Meat Science*, 17(2), 107-138.
- Kim, E. J., Scollan, N. D., Richardson, I., Gibson, K., Ball, R., & Coulmier, K. (2009). Effect of lipid-rich plant extract on the fatty acids composition and meat quality of Charolais × Friesian steers. In *Advances in Animal Biosciences*. Southport, UK: British Society of Animal Science.
- Latham, M. J., Storry, J. E., & Sharpe, M. E. (1972). Effect of Low-Roughage Diets on the Microflora and Lipid Metabolism in the Rumen. *Applied Microbiology*, 24(6), 871-877.
- Lindqvist, H., Nadeau, E., & Jensen, S. K. (2012). Alpha-tocopherol and β -carotene in legume–grass mixtures as influenced by wilting, ensiling and type of silage additive. *Grass and Forage Science*, 67(1), 119-128.
- Liu, Q., Scheller, K. K., Arp, S. C., Schaefer, D. M., & Williams, S. N. (1996). Titration of fresh meat color stability and malondialdehyde development with Holstein steers fed vitamin E-supplemented diets. *Journal of Animal Science*, 74(1), 117-126.
- Lopez-Huertas, E. (2010). Health effects of oleic acid and long chain omega-3 fatty acids (EPA and DHA) enriched milks. A review of intervention studies. *Pharmacological Research*, 61(3), 200-207.

- Macdougall, D. B. (1982). Changes in the Color and Opacity of Meat. *Food Chemistry*, 9(1-2), 75-88.
- MacFie, HJ, Bratchell, N, Greenhoff, K, Vallis, LV (1989) Designs to balance the effect of order of presentation and first-order carry-over effects in hall tests. *Journal of Sensory Studies*, 4, 129-148.
- Mahecha, L., Dannenberger, D., Nuernberg, K., Nuernberg, G., Hagemann, E., & Martin, J. (2010). Relationship between lipid peroxidation and antioxidant status in the muscle of German Holstein bulls Fed n-3 and n-6 PUFA-enriched diets. *Journal of Agricultural and Food Chemistry*, 58(14), 8407-8413.
- Mapiye, C., Turner, T. D., Rolland, D. C., Basarab, J. A., Baron, V. S., McAllister, T. A., Block, H. C., Uttaro, B., Aalhus, J. L., & Dugan, M. E. R. (2013). Adipose tissue and muscle fatty acid profiles of steers fed red clover silage with and without flaxseed. *Livestock Science*, 151, 11–20.
- Marmer, W. N., Maxwell, R. J., & Williams, J. E. (1984). Effects of Dietary Regimen and Tissue Site on Bovine Fatty-Acid Profiles. *Journal of Animal Science*, 59(1), 109-121.
- McAfee, A. J., McSorley, E. M., Cuskelly, G. J., Moss, B. W., Wallace, J. M. W., Bonham, M. P., & Fearon, A. M. (2010). Red meat consumption: An overview of the risks and benefits. *Meat Science*, 84(1), 1-13.
- Nikolova, H. D., & Inman, J. J. (2015). Healthy Choice: The Effect of Simplified Point-of-Sale Nutritional Information on Consumer Food Choice Behavior. *Journal of Marketing Research (JMR)*, 52(6), 817-835.
- Nuernberg, K., Dannenberger, D., Nuernberg, G., Ender, K., Voigt, J., Scollan, N. D., Wood, J. D., Nute, G. R., & Richardson, R. I. (2005). Effect of a grass-based and a concentrate feeding system on meat quality characteristics and fatty acid composition of longissimus muscle in different cattle breeds. *Livestock Production Science*, 94(1-2), 137-147.
- Nuernberg, K., Nuernberg, G., Ender, K., Lorenz, S., Winkler, K., Rickert, R., & Steinhart, H. (2002). N-3 fatty acids and conjugated linoleic acids of longissimus muscle in beef cattle. *European Journal of Lipid Science and Technology*, 104(8), 463-471.
- Payne, R. W., Murray, D. A., Harding, S. A., Baird, D. B., & Soutar, D. M. (2011). *Introduction to GenStat for Windows™* (14th Edition ed.). Hemel Hempstead, UK: VSN International.
- Pocklington WD and Dieffenbacher A (1988). Determination of tocopherols and tocotrienols in vegetable oils and fats by high performance liquid chromatography: results of a

- collaborative study and the standardised method. *Pure and Applied Chemistry*, 60, 877–892.
- Priolo, A., Micol, D., & Agabriel, J. (2001). Effects of grass feeding systems on ruminant meat colour and flavour. A review. *Animal Research*, 50(3), 185-200.
- Rechulicz, J., Ognik, K., & Grela, E. R. (2014). The Effect of Adding Protein-Xanthophylls Concentrate (PX) from lucerne (*Medicago sativa*) on Growth Parameters and Redox Profile in Muscles of carp, *Cyprinus carpio* (L.). *Turkish Journal of Fisheries and Aquatic Sciences*, 14(3), 697-703.
- Russo, G. L. (2009). Dietary n-6 and n-3 polyunsaturated fatty acids: From biochemistry to clinical implications in cardiovascular prevention. *Biochemical Pharmacology*, 77(6), 937-946.
- Salter, A. M. (2013). Dietary fatty acids and cardiovascular disease. *Animal*, 7, 163-171.
- Scollan, N., Hocquette, J. F., Nuernberg, K., Dannenberger, D., Richardson, I., & Moloney, A. (2006). Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. *Meat Science*, 74(1), 17-33.
- Scollan, N. D., Choi, N.-J., Kurt, E., Fisher, A. V., Enser, M., & Wood, J. D. (2001). Manipulating the fatty acid composition of muscle and adipose tissue in beef cattle. *British Journal of Nutrition*, 85(01), 115-124.
- Scollan, N. D., Dannenberger, D., Nuernberg, K., Richardson, I., MacKintosh, S. B., Hocquette, J. F., & Moloney, A. P. (2014). Enhancing the nutritional and health value of beef lipids and their relationship with meat quality. *Meat Science*, 97(3), 384-394.
- Scollan, N. D., Richardson, I., Smet, S. d., Moloney, A. P., Doreau, M., Bauchart, D., & Nuernberg, K. (2005). Enhancing the content of beneficial fatty acids in beef and consequences for meat quality. In *EAAP Publication No.112* (pp. 151-162). Wageningen: Wageningen Academic Publishers.
- Shen, X. Z., Dannenberger, D., Nuernberg, K., Nuernberg, G., & Zhao, R. Q. (2011). Trans-18:1 and CLA Isomers in Rumen and Duodenal Digesta of Bulls Fed n-3 and n-6 PUFA-Based Diets. *Lipids*, 46(9), 831-841.
- Shingfield, K. J., Bonnet, M., & Scollan, N. D. (2013). Recent developments in altering the fatty acid composition of ruminant-derived foods. *Animal*, 7, 132-162.
- Siri-Tarino, P. W., Sun, Q., Hu, F. B., & Krauss, R. M. (2010). Saturated fat, carbohydrate, and cardiovascular disease. *American Journal of Clinical Nutrition*, 91(3), 502-509.

- Sitz, B. M., Calkins, C. R., Feuz, D. M., Umberger, W. J., & Eskridge, K. M. (2005). Consumer sensory acceptance and value of domestic, Canadian, and Australian grass-fed beef steaks¹. *Journal of Animal Science*, 83(12), 2863-2868.
- Tarladgis, B. G., Watts, B. M., Younathan, M. T., & Dugan, L. (1960). A Distillation Method for the Quantitative Determination of Malonaldehyde in Rancid Foods. *Journal of the American Oil Chemists Society*, 37(1), 44-48.
- Van Nevel, C. J., & Demeyer, D. I. (1996). Influence of pH on lipolysis and biohydrogenation of soybean oil by rumen contents in vitro. *Reproduction Nutrition Development*, 36(1), 53-63.
- Vatansever, L., Kurt, E., Enser, M., Nute, G. R., Scollan, N. D., Wood, J. D., & Richardson, R. I. (2000). Shelf life and eating quality of beef from cattle of different breeds given diets differing in n-3 polyunsaturated fatty acid composition. *Animal Science*, 71, 471-482.
- Warren, H. E., Scollan, N. D., Enser, M., Hughes, S. I., Richardson, R. I., & Wood, J. D. (2008). Effects of breed and a concentrate or grass silage diet on beef quality in cattle of 3 ages. I: Animal performance, carcass quality and muscle fatty acid composition. *Meat Science*, 78(3), 256-269.
- Warren, H. E., Scollan, N. D., Nute, G. R., Hughes, S. I., Wood, J. D., & Richardson, R. I. (2008). Effects of breed and a concentrate or grass silage diet on beef quality in cattle of 3 ages. II: Meat stability and flavour. *Meat Science*, 78(3), 270-278.
- Waters, S.M., Kelly, J.P., O'Boyle, P., Moloney, A.P. and Kenny, D.A. (2009). Effect of level and duration of dietary n-3 polyunsaturated fatty acid supplementation on the transcriptional regulation of Δ^9 -desaturase in muscle of beef cattle. *Journal of Animal Science*, 87, 244-252.
- Williams, P. (2007). Nutritional composition of red meat. *Nutrition & Dietetics*, 64, S113-S119.
- Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., Hughes, S. I., & Whittington, F. M. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Science*, 78(4), 343-358.
- World Health Organisation (WHO). (2003). Diet, nutrition and the prevention of chronic diseases. Report of the joint WHO/FAO expert consultation. In *WHO Technical Report Series* (Vol. 916). Geneva.

Figure 1. The change in colour chroma (mean $\pm$ sem) over 20 days simulated retail display of *M. longissimus* steaks in modified atmosphere from Belgian Blue cattle fed straw + control concentrates (S-CC), straw + PX-concentrate (S-PXC), grass silage (GS), silage + 75 g PX/kg silage DMI (GS-LPX), or silage + 150 g PX/kg silage DMI (GS-HPX). A chroma value of 18 indicates the threshold for consumer acceptability (Hood and Riordan, 1973; MacDougall, 1982).

Table 1. Formulation of experimental concentrate diet (kg/tonne fresh).

	Control Concentrate	PX Concentrates
Barley	264	283
Wheatfeed	251	296
GM Soya Hipro	262	-
PX meal	-	225
Sugar beet pulp	98	100
Limestone flour	12	8
Salt	3	3
Sodium bicarbonate	1	1
Ammonium chloride	3	3
Wynnstay cattle supplement	3	3
Vitamin E (111 IU/g)	-	18
Molasses	60	60
Megalac	23	-
Non GM fat	20	-

Table 2. Chemical composition and fatty acid composition (g/kg dry matter, unless otherwise stated) in experimental feeds.

	Feed				
	Control Concentrates	PX Concentrates	PX Crumb	Straw	Silage
<i>Chemical composition</i>					
Dry matter ^a	879.4	878.9	924.7	842.7	364.8
Organic matter	928.4	904.5	876.5	935.8	921.4
Total nitrogen (TN)	37.4	36.6	85.8	7.9	24.9
Water-soluble carbohydrates (WSC)	92.1	62.5	20.8	15.1	125.2
Neutral detergent fibre (NDF)	217.8	237.2	N.D.	807.7	499.2
Acid detergent fibre (ADF)	93.2	82.5	39.0	521.4	308.7
Ether extract (EE)	64.3	64.1	137.0	9.4	38.8
Ammonia nitrogen ^b	-	-	-	-	85.4
pH	-	-	-	-	3.92
<i>Fatty acid composition</i>					
Total	72.2	47.4	86.0	4.2	25.6
12:0	0.6	0.3	0.5	0.06	0.1
14:0	0.6	0.8	2.0	0.3	0.2
16:0	22.0	9.2	15.5	1.2	4.5
16:1	0.14	0.14	0.24	0.03	0.12
18:0	2.1	0.8	2.1	0.1	0.3
18:1 ^{cis} -9	18.6	5.0	2.7	0.2	0.5
18:1 ^{cis} -11	0.7	0.4	0.3	0.01	0.1
18:2 ⁿ -6	23.0	18.0	14.8	0.6	3.4
18:3 ⁿ -3	3.4	11.1	42.5	0.7	14.7
20:0	0.18	0.15	0.40	0.15	0.11

^a (g/kg fresh matter). ^b (g/kg total nitrogen). N.D. not determined.

1 Table 3. Animal performance and carcass classification of Belgian Blue cattle fed straw + control concentrates (S-CC), straw + PX-concentrate (S-PXC), grass
2 silage (GS), silage + 75 g PX/kg silage DMI (GS-LPX), or silage + 150 g PX/kg silage DMI (GS-HPX).

	Diet					S.E.D.	Probability
	S-CC	S-PXC	GS	GS-LPX	GS-HPX		
Total intake ^a	10.5 ^q	10.6 ^q	8.3 ^r	8.9 ^r	8.7 ^r	0.32	<0.001
Concentrate intake ^a	6.8	6.8	-	-	-	0.11	0.916
PX intake ^a	-	1.7 ^{q†}	-	0.6 ^{r††}	1.1 ^{s††}	0.03	<0.001
Forage intake ^a	3.6 ^q	3.8 ^q	8.3 ^r	8.3 ^r	7.7 ^r	0.29	<0.001
Live weight gain (kg/d) ^b	1.21	1.06	1.02	1.11	1.08	0.093	0.392
Age at slaughter (d)	627	626	622	624	624	9.3	0.989
Half carcass weight (kg) ^b	178.0	163.5	170.9	172.9	170.0	5.90	0.205
Conformation ^c	73.8	73.8	77.5	85.0	73.8	6.77	0.399
Fatness ^c	55.6	46.9	61.2	63.8	55.0	8.15	0.294
pH 3 h	6.09	6.05	6.02	5.92	6.05	0.108	0.575
pH 48 h	5.55	5.68	5.59	5.54	5.54	0.052	0.045
Drip loss (%)	2.0	2.4	1.3	1.7	1.9	0.369	0.087

3 ^a Intakes expressed as kg DM/day.

4 ^b Half carcass cold weight.

5 ^c Based on EEC beef carcass classification scheme as amended by Kempster, Cook, & Grantleysmith (1986).

6 [†] PX consumed calculated using concentration in the PXC pellet and amount of PXC concentrate consumed.

7 ^{qrstu} Means with different superscript differ significantly, within row, P<0.05; using Tukey's *post hoc*.

8

9 Table 4. Average daily intakes (g/day, DM basis) of total and selected fatty acids by steers fed straw + control concentrates (S-CC), straw + PX-concentrate
10 (S-PXC), grass silage (GS), silage + 75 g PX/kg silage DMI (GS-LPX), or silage + 150 g PX/kg silage DMI (GS-HPX).
11

	Diet					S.E.D.	Probability
	S-CC	S-PXC	GS	GS-LPX	GS-HPX		
Total	507.7 ^q	338.6 ^r	212.0 ^s	261.5 ^t	287.9 ^u	8.35	<0.001
12:0	4.4 ^q	2.5 ^r	0.8 ^s	1.1 ^t	1.3 ^u	0.05	<0.001
14:0	5.2 ^q	6.3 ^r	1.4 ^s	2.5 ^t	3.4 ^u	0.11	<0.001
16:0	154.5 ^q	67.3 ^r	36.9 ^s	45.8 ^t	50.6 ^t	1.74	<0.001
16:1	1.0 ^q	1.0 ^q	1.0 ^q	1.2 ^r	1.2 ^r	0.03	<0.001
18:0	15.1 ^q	6.3 ^r	2.8 ^s	3.9 ^t	4.7 ^u	0.16	<0.001
18:1 ^{cis} -9	127.3 ^q	34.6 ^r	3.9 ^s	5.5 ^s	6.5 ^s	0.95	<0.001
18:1 ^{cis} -11	4.5 ^q	2.5 ^r	0.8 ^s	0.9 ^t	1.0 ^t	0.04	<0.001
18:2 ⁿ -6	158.8 ^q	124.6 ^r	28.4 ^s	36.9 ^t	42.0 ^u	1.75	<0.001
18:3 ⁿ -3	25.4 ^q	78.2 ^r	121.6 ^s	146.2 ^t	158.0 ^u	4.00	<0.001
20:0	1.8 ^q	1.6 ^r	0.9 ^s	1.2 ^t	1.3 ^t	0.05	<0.001

^{qrst} Means with different superscript differ significantly, within row (P<0.05).

Table 5. A summary of the concentrations (mg/100 g) and proportions (times 100) of fatty acid classes and health indices in total lipid of the *M. longissimus* of Belgian Blue cattle fed straw + control concentrates (S-CC), straw + PX-concentrate (S-PXC), grass silage (GS), silage + 75 g PX/kg silage DMI (GS-LPX), or silage + 150 g PX/kg silage DMI (GS-HPX).

	Diet					S.E.D.	Probability
	S-CC	S-PXC	GS	GS-LPX	GS-HPX		
Total lipid	2531.6	1999.0	2551.3	2510.4	2433.2	379.3	0.568
Total NL	2028.0	1542.5	2066.6	2021.3	1930.9	363.7	0.595
Total PL	503.7	456.5	484.7	489.1	502.3	22.3	0.235
SFA ^a	1059.4	806.1	1054.9	1012.9	990.1	174.9	0.593
MUFA ^b	1017.3	729.4	1061.3	1031.0	966.1	163.2	0.272
PUFA ^c	220.6 ^{qr}	250.8 ^r	163.5 ^s	192.5 ^{qs}	205.7 ^q	13.6	<0.001
Sum <i>n</i> -6 ^d	174.9 ^q	179.0 ^q	91.4 ^r	105.6 ^r	111.6 ^r	9.2	<0.001
Sum <i>n</i> -3 ^e	45.7 ^q	71.8 ^r	72.1 ^r	86.9 ^{rs}	94.1 ^s	5.3	<0.001
<i>Proportions (%)</i>							
SFA	41.6	39.8	41.2	40.2	40.1	1.1	0.459
MUFA	40.0 ^q	36.0 ^r	41.5 ^q	40.8 ^q	39.5 ^q	1.1	<0.001
PUFA	9.1 ^q	13.3 ^r	6.7 ^q	7.9 ^q	9.1 ^q	1.1	<0.001
Sum <i>n</i> -6 ^d	7.2 ^q	9.5 ^r	3.7 ^s	4.4 ^s	4.9 ^s	0.7	<0.001
Sum <i>n</i> -3 ^e	1.9 ^q	3.8 ^{rs}	3.0 ^r	3.6 ^{rs}	4.2 ^s	0.4	<0.001
<i>Nutritional Indices</i>							
P:S ^f	0.13 ^q	0.23 ^r	0.08 ^q	0.11 ^q	0.13 ^q	0.02	<0.001
<i>n</i> -6: <i>n</i> -3 ^{d,e}	3.86 ^q	2.50 ^r	1.26 ^s	1.22 ^s	1.19 ^s	0.10	<0.001
EPA+DHA ^g	11.35 ^q	13.93 ^{qr}	16.71 ^{rs}	18.41 st	21.11 ^t	1.24	<0.001

^{qrstu} Means with different superscript differ significantly, within row (P<0.05).

^a SFA, saturated fatty acids (12:0 + 14:0 + 16:0 + 18:0).

^b MUFA, monounsaturated fatty acids (16:1 + 18:1 + 20:1).

^c PUFA, polyunsaturated fatty acids (18:2*n*-6 + 18:3*n*-3 + 20:3*n*-6 + 20:4*n*-6 + 20:5*n*-3 + 22:4*n*-6 + 22:5*n*-3 + 22:6*n*-3).

^d *n*-6 PUFA (18:2*n*-6 + 20:3*n*-6 + 20:4*n*-6 + 22:4*n*-6).

^e *n*-3 PUFA (18:3*n*-3 + 20:4*n*-3 + 20:5*n*-3 + 22:5*n*-3 + 22:6*n*-3).

^f P:S, (18:2*n*-6 + 18:3*n*-3)/(12:0 + 14:0 + 16:0 + 18:0).

^g EPA (20:5*n*-3) + DHA (22:6*n*-3), mg/100g muscle.

27 Table 6. Fatty acid concentration (mg/100 g muscle) in the neutral lipid and phospholipid of *M. longissimus* from Belgian Blue cattle fed straw + control
 28 concentrates (S-CC), straw + PX-concentrate (S-PXC), grass silage (GS), silage + 75 g PX/kg silage DMI (GS-LPX), or silage + 150 g PX/kg silage DMI (GS-HPX).

	Diet					S.E.D.	Probability
	S-CC	S-PXC	GS	GS-LPX	GS-HPX		
Neutral Lipid							
14:0	59.4	41.6	62.7	56.0	52.3	11.5	0.417
16:0	577.4	424.2	595.0	553.4	525.4	104.6	0.517
16:1	78.7	55.2	86.3	80.0	67.4	14.2	0.223
18:0	293.9	228.8	278.4	283.1	289.6	55.8	0.774
18:1 <i>trans</i>	71.8	59.8	52.0	58.5	64.9	12.2	0.573
18:1 <i>cis</i> -9	734.7	533.3	766.3	753.7	697.5	131.2	0.395
18:1 <i>cis</i> -11	20.0	15.3	20.6	20.9	18.0	3.01	0.353
18:2 <i>n</i> -6	29.4 ^{qr}	32.9 ^r	15.9 ^s	18.6 ^{qs}	20.0 ^{qs}	4.0	<0.001
20:1	2.1	1.5	1.8	2.0	1.7	0.3	0.419
18:3 <i>n</i> -3	6.5 ^q	15.5 ^r	9.3 ^{qs}	13.6 ^{rs}	15.2 ^r	2.0	<0.001
Total	2028.0	1542.5	2066.6	2021.3	1930.9	363.7	0.595
Phospholipid							
14:0	1.2	0.8	1.3	1.1	1.2	0.2	0.309
16:0	76.1	64.3	70.0	69.7	71.0	3.9	0.080
16:1	6.6 ^q	3.8 ^r	8.9 ^s	7.2 ^q	7.4 ^{qs}	0.5	<0.001
18:0	50.3	45.5	46.4	48.8	49.8	2.1	0.128
18:1 <i>trans</i>	3.9 ^q	3.5 ^{qr}	3.1 ^r	3.6 ^{qr}	4.0 ^q	0.3	0.019
18:1 <i>cis</i> -9	90.1 ^q	47.7 ^r	113.3 ^s	96.2 ^{qs}	96.3 ^{qs}	6.2	<0.001
18:1 <i>cis</i> -11	9.2	8.9	8.7	8.8	8.7	0.5	0.900
18:2 <i>n</i> -6	91.9 ^q	101.6 ^q	40.8 ^r	51.5 ^r	52.7 ^r	4.4	<0.001
18:3 <i>n</i> -3	7.3 ^q	20.2 ^{rs}	17.7 ^r	24.7 st	26.1 ^t	1.7	<0.001
20:3 <i>n</i> -6	11.6 ^q	8.8 ^r	5.0 ^s	5.6 ^s	6.3 ^s	0.5	<0.001
20:4 <i>n</i> -6	37.3 ^q	32.2 ^r	27.0 ^s	27.2 ^s	29.7 ^{sr}	1.7	<0.001
20:4 <i>n</i> -3	2.0 ^q	2.9 ^q	4.2 ^r	4.8 ^r	5.2 ^r	0.4	<0.001
20:5 <i>n</i> -3	9.7 ^q	12.2 ^{qr}	14.1 ^{rs}	15.9 st	18.1 ^t	1.1	<0.001
22:4 <i>n</i> -6	3.2 ^q	2.0 ^r	1.9 ^r	1.8 ^r	1.9 ^r	0.2	<0.001
22:5 <i>n</i> -3	16.7 ^q	17.3 ^q	21.5 ^r	22.9 ^r	23.7 ^r	1.1	<0.001
22:6 <i>n</i> -3	1.6 ^q	1.6 ^q	2.4 ^r	2.3 ^r	2.8 ^r	0.2	<0.001

Total	503.7	456.5	484.7	489.1	502.3	22.3	0.235
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Means with different superscript differ significantly, within row (P<0.05).

Table 7. The concentration (mg/100 g muscle) of individual CLA isomers in the total lipid fraction of the *M. longissimus* from Belgian Blue cattle fed straw + control concentrates (S-CC), straw + PX-concentrate (S-PXC), grass silage (GS), silage + 75 g PX/kg silage DMI (GS-LPX), or silage + 150 g PX/kg silage DMI (GS-HPX).

	Diet					S.E.D.	Probability
	S-CC	S-PXC	GS	GS-LPX	GS-HPX		
CLAttr12,tr14	0.33	0.32	0.20	0.23	0.26	0.10	0.685
CLAttr11,tr13	0.38 ^q	0.42 ^{qr}	0.55 ^{qr}	0.62 ^r	0.54 ^{qr}	0.08	0.023
CLAttr10,tr12	0.04	0.06	0.03	0.04	0.04	0.01	0.373
CLAttr9,tr11	0.28	0.29	0.28	0.31	0.35	0.05	0.630
CLAttr8,tr10	0.04	0.04	0.03	0.02	0.02	0.01	0.119
CLAttr7,tr9	0.03	0.02	0.02	0.03	0.02	0.01	0.689
CLAc12,tr14 ^a	0.04	0.11	0.05	0.05	0.04	0.03	0.066
CLAttr11,c13	1.82 ^q	3.35 ^{qr}	3.82 ^{qr}	4.86 ^r	5.30 ^r	0.81	0.001
CLAc11,tr13	0.20	0.19	0.21	0.24	0.31	0.07	0.390
CLAttr10,c12 ^a	0.21	0.13	0.15	0.13	0.15	0.04	0.272
CLAc9,tr11 ^a	15.91	15.85	16.18	16.96	16.07	2.6	0.993
CLAttr8,c10 ^a	0.11	0.22	0.15	0.11	0.13	0.07	0.464
CLAttr7,c9 ^a	2.47	2.05	2.07	1.78	1.95	0.33	0.323

Means with different superscript differ significantly, within row, P<0.05.

^a Sum of the *cis,trans* and the corresponding *trans,cis* isomer.

43 Table 8. Colour saturation, lipid oxidation and Vitamin E concentration *M. longissimus* from Belgian Blue cattle fed straw + control concentrates (S-CC),
 44 straw + PX-concentrate (S-PXC), grass silage (GS), silage + 75 g PX/kg silage DMI (GS-LPX), or silage + 150 g PX/kg silage DMI (GS-HPX).

	Diet					S.E.D.	Probability
	S-CC	S-PXC	GS	GS-LPX	GS-HPX		
<i>Bloomed Colour</i>							
<i>L</i> [*]	40.28	39.96	40.33	40.45	40.11	1.242	0.996
<i>a</i> [*]	20.83 ^q	19.22 ^{qr}	19.76 ^{qr}	20.39 ^{qr}	17.96 ^r	0.890	0.026
<i>b</i> [*]	9.58 ^q	8.88 ^{qr}	9.00 ^{qr}	9.60 ^q	7.53 ^r	0.627	0.014
Hue	24.62 ^q	24.74 ^q	24.43 ^{qr}	25.19 ^q	22.51 ^r	0.734	0.009
Chroma	22.93 ^q	21.18 ^{qr}	21.72 ^{qr}	22.54 ^q	19.49 ^r	1.054	0.022
<i>Lipid Oxidation</i>							
Day 10 ^{a,b}	1.19 ^q	0.27 ^r	0.55 ^s	0.76 ^{qs}	1.25 ^q	0.098	<0.001
Vitamin E ^c	3.2 ^q	7.7 ^r	3.9 ^{qs}	4.4 ^s	4.2 ^{qs}	0.373	<0.001
<i>Colour</i>							
days to a chroma value of 18	16.5 ^q	18.9 ^r	16.62 ^q	17.4 ^{qr}	16.4 ^q	0.86	0.033

45 ^{qrstu} Means with different superscript differ significantly, within row.

46 ^a mg malonaldehyde/kg meat.

47 ^b Values are geometric means from back-transformations with the S.E.D. of log₁₀ values (Log₁₀ transformations used for analysis).

48 ^c mg/kg muscle.

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51 Table 9. Eating quality of grilled beef loin steak, cooked to 74°C internal endpoint temperature, from Belgian Blue cattle fed control concentrates (S-CC),
52 straw + PX-concentrate (S-PXC), grass silage (GS), silage + 75 g PX/kg silage DMI (GS-LPX), or silage + 150 g PX/kg silage DMI (GS-HPX). Values are the means
53 derived from analysis of variance with diet and assessor as factors, with 8 replicates.

Attribute	Diet					S.E.D.	Probability
	S-CC	S-PXC	GS	GS-LPX	GS-HPX		
<i>8 point category scale</i>							
Texture	4.51 ^q	4.57 ^q	3.89 ^r	4.55 ^q	4.55 ^q	0.196	<0.001
Juiciness	4.5	4.2	4.6	4.4	4.6	0.17	0.09
Beef flavour intensity	4.6	4.8	4.5	4.9	4.9	0.20	0.18
Abnormal flavour intensity	2.9	2.8	3.0	2.7	2.5	0.22	0.26
<i>100mm line scale</i>							
Greasy	12.53 ^{qr}	11.12 ^r	15.54 ^q	12.76 ^{qr}	13.17 ^{qr}	1.372	0.03
Bloody	6.12 ^{qr}	4.32 ^r	10.06 ^q	6.81 ^{qr}	4.49 ^{qr}	1.844	0.04
Livery	14.0	14.5	16.3	14.6	13.8	1.94	0.72
Metallic	17.6	17.3	18.8	18.3	19.8	2.09	0.76
Bitter	8.3	7.8	11.2	7.3	8.1	2.05	0.34
Sweet	11.9	11.3	12.9	11.9	11.0	1.33	0.67
Rancid	2.1	2.8	4.5	2.4	0.8	1.65	0.25
Fishy	6.6	4.7	6.6	5.4	5.0	1.30	0.43
Acidic	13.6	15.5	15.3	14.2	16.2	2.54	0.86
Cardboard	20.7	18.2	22.6	19.0	19.0	2.37	0.36
Vegetable/Grass	14.3	11.7	14.8	15.7	14.4	2.18	0.45
Dairy	12.4	11.3	14.0	12.7	13.8	1.57	0.42
<i>Hedonic</i>							
Overall liking	44.9	45.9	44.7	48.5	50.5	2.79	0.17

^{qr} Means with different superscript differ significantly, within row (P<0.05).