



End of Project Report

ENHANCEMENT OF THE NUTRITIONAL VALUE AND EATING QUALITY OF BEEF

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1. SUMMARY/CONCLUSIONS

Consumer interest in the nutritional aspects of health has increased interest in developing methods to manipulate the fatty acid composition of ruminant products. Ruminant meats such as beef and lamb are often criticised by nutritionists for having high amounts of saturated fatty acids (S) and low polyunsaturated fatty acids (P). The P:S ratio in beef is approximately 0.1, the ideal being about 0.4.

This project is part of a larger EU-supported project entitled Healthy Beef (Enhancing the content of beneficial fatty acids in beef and improving meat quality for the consumer: QLRT-CT-2000-31423).

The Teagasc contribution, which was a collaboration between Grange Research Centre and The National Food Centre, focussed on nutritional manipulation of beef cattle. In particular, on exploiting grazing and fishoil as tools to enhance the concentration of "healthy" fatty acids in beef.

The conclusions were:

- The beneficial effect of a grazed grass-based diet on the fatty acid composition of beef was confirmed
- The scale of this beneficial effect is strongly dependent on the duration of grazing
- The optimum concentration of beneficial fatty acids was not achieved suggesting that feeding management prior to grazing is important
- Grazing influenced beef colour and drip-loss in a durationdependent manner
- Animals finished off grass for 40 or 98 days produced meat that was tougher than that from animals finished on silage and concentrates or fed grass for the last 158 days.
- Fish oil supplementation enhanced the concentration in beef, of fatty acids that are beneficial to human health
- The linear response to increasing level of fish oil consumption indicates scope to further enhance the concentrations of beneficial fatty acids in beef



- Wilting of grass prior to ensiling did not impact negatively on the overall content of n-3P in muscle, but it increased the concentration of conjugated linoleic acid
- Dietary inclusion of fish oil or wilting of grass prior to ensiling did not affect muscle appearance
- Fish oil seemed to increase tenderness but only at the high level of inclusion. This merits further study
- There was some evidence that wilting of grass prior to ensiling enhanced meat tenderness. This needs to be confirmed.





2. GENERAL INTRODUCTION

The relationships between dietary fat and the incidence of lifestyle diseases, particularly coronary heart disease are well established. Consequently it is suggested that the contribution of fat and saturated fatty acids (SFA) to dietary energy intake should not exceed 0.35 and 0.10 of total intake, respectively, the ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA) (P:S ratio) should be around 0.45 and the ratio of n-6 to n-3 PUFA should be less than 4. Although it is the fat content and fatty acid composition of the whole diet which is important, research has focused on changing individual foods to be more in line with these guidelines.

Ruminant meats such as beef and lamb are often criticised by nutritionists for having high amounts of SFA and low PUFA. The P:S ratio in beef is approximately 0.1. In contrast, the ratio of n-6:n-3 PUFA is beneficially low, at approximately 2.0 This reflects the considerable amounts of n-3 PUFA in beef, particularly α -linolenic (18:3n-3) and the long chain PUFA, eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3). Meat, fish, fish oils and eggs are the only significant sources of these n-3 C20 PUFA for man. Although meat has lower concentration of these fatty acids compared to oily fish, it is a very significant source for many people, since fish consumption is low. Ruminants also produce conjugated linoleic acid (CLA) which offers a range of nutritional benefits..

This project is part of a larger EU support project entitled Healthy Beef (Enhancing the content of beneficial fatty acids in beef and improving meat quality for the consumer: QLRT-CT-2000-3 1423). The project examined strategies (nutritional, genetic) to enhance the nutritional value of beef by having higher quantities of n-3 PUFA and CLA. Since strategies that enhance the beneficial fatty acids in beef must not have a deleterious impact on other aspects of quality, the implications for meat quality, in particular colour shelf life and sensory attributes were also examined. The role of the rumen in manipulating the composition of dietary fatty acids was also studied. The Teagasc contribution to this project focussed on nutritional manipulation of beef cattle.



3. EXPERIMENT 1:

Influence of duration of grazing, prior to slaughter, on the fatty acid composition of intramuscular and subcutaneous adipose tissue of beef heifers

Introduction

Feed costs are a major portion of total variable costs in beef production systems. Grazed grass is often the cheapest feedstuff in temperate climates. In addition to decreasing the cost of beef production, beef from cattle produced from grass has a higher concentration of fatty acids considered to be beneficial to human health than beef produced from more intensive production systems. Little information is available on the time required for grazing animals to achieve the maximum concentrations of beneficial fatty acids in tissue. Accordingly, the first objective of this experiment was to determine the fatty acid concentrations in muscle and adipose tissue of cattle produced from a standard Irish grass silage and concentrates finishing system but allowed to graze grass for different periods prior to slaughter. The second objective was to determine the impact of these modifications to the production system on selected quality characteristics of the resulting beef.

Materials and Methods Animal Management

Sixty crossbred continental heifers (initial mean live weight of 338 kg, SD = 39.73 kg and approximately 12 months of age) were assigned in a randomised complete block design to one of four dietary treatments, i.e. ad libitum unwilted grass silage and concentrates for 158 days; ad libitum unwilted grass silage and concentrates for 118 days, followed by 40 days of grazing; ad libitum unwilted grass silage and concentrates for silage and concentrates for 59 days, followed by 99 days of grazing; or grazed grass for 158 days. Animals assigned to the indoor rations were penned in a slatted floor shed, in groups of five animals according to descending order of block. The initial daily ration was 3kg of



concentrates plus fresh silage. The concentrate allowance was adjusted based on increasing bodyweight to maintain a similar forage to concentrate ratio in the ration. Animals assigned to longterm grazing, were managed as groups of five animals each. They were offered an initial daily grass allowance of 132kg dry matter (DM) for the whole group.

The duration of the feeding period was 158 days. The animals were then transported to a commercial slaughter facility and slaughtered within 1h of arrival. Following slaughter, carcasses were assessed for conformation and fatness score ([S]EUROP scale). The weight of the carcass and of the peri-renal fat depot were recorded and carcasses were hung by the Achilles tendon for 24h at a chilling temperature of 4° C. Longissimus dorsi muscle was then excised, transported to The National Food Centre and stored overnight at 4° C. At 48h post-mortem, Longissimus dorsi muscle pH, drip loss and colour (HunterLab L (lightness), a (redness), and b (yellowness)) and subcutaneous fat (HunterLab L, a, b) colour were measured. Duplicate samples of Longissimus dorsi (25mm thick) were collected from the 5th rib region and a sample of subcutaneous fat from the 9th rib region of the Longissimus dorsi were quick frozen and stored at -30°C for measurement of fatty acid composition at Grange Research Centre. A sample of Longissimus dorsi was collected, aged for 14 days and stored frozen prior to sensory analysis in The National Food Centre. Additional samples of Longissimus dorsi were aged for 2, 7 and 14 days and stored frozen pending measurement of shear force. A further 2 day-aged sample was frozen prior to chemical analysis.

Chemical analysis.

Muscle pH was measured using a portable pH meter. Warner Bratzler shear force, an instrumental measure of tenderness, was measured on 1 freshly cut steak (2.54 cm thick) from the posterior end of the LD muscles after cooking to a core temperature of 70°C. For compositional analysis, frozen samples (~180g) were thawed and homogenised prior to the application of standard methods. Drip loss was determined by weight loss due to gravity acting on freshly cut muscle samples (2.54 cm thick and approximately 100g

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weight) hung for 96 hours. Sensory analysis was performed by an eight-membered, in-house trained panel on steaks grilled to an internal temperature of 70°C, according to the AMSA (American Meat Science Association) Guidelines for Cookery and Sensory Evaluation of Meat (AMSA 1995).

For fatty acid analysis, intramuscular adipose tissue was extracted from homogenised muscle using 2:1 chloroform/methanol solution with 0.05% (w/v) of butylated hydroxytoluene as antioxidant. The extracted adipose tissue was separated into neutral and polar lipid fractions, with SPE cartridges NH₂ (Bond-Elut 500mg, 3ml reservoir, Varian, Palo Alto, CA, United States). The separated lipid classes were then dissolved in 300ml of toluene for the preparation of fatty acid methyl esters (FAME). The methylation procedure used was a combination of alkaline and acidic transesterification, using tricosanoic acid methanol (C23:0) as internal standard. The FAME obtained were analysed by gas chromatography (GC) using a Varian 3800 GC equipped with a CP-Sil 88 capillary column (100m. 32mm i.d., 0.25mm film thickness, Chrompack, The Netherlands) and a Varian 8400 autosampler. The injector and the FID were kept at a constant temperature of 250°C and 260°C respectively. The column oven was kept at an initial temperature of 40°C, held for 2min, and the temperature increased at a rate of 20°C/min up to 80°C, held for 2min, increased to 160°C at 20°C/min and increased to 220°C at 4°C/min, then increased up to 240°C at 2°C/min and held for 8min. for a total run time of 43 minutes. Hydrogen was used as carrier gas. For peak identification a standard mix of 37 FAME (Supelco Inc., Bellefonte, PA, United States) was used.

The fatty acid composition of feedstuffs was determined by using the procedure described by Sukhija and Palmquist (1988) and purifying the samples with activated charcoal. The samples extracted in toluene were analysed by GC as described above.

Statistical Analysis

Data were were analysed using analysis of variance procedures with treatment and block as main effects. The relationship between the duration of grazing and response variables was investigated using



orthogonal polynomials. For sensory data, means for each trait for each sample were calculated and analysed using the above model. For fatty acid data, means were calculated across duplicate samples and statistically analysed as described above.

Results

Animal performance

Feed composition data are reported in Table 1. Grass and grass silage were characterised by a higher PUFA and a lower SFA concentration than the concentrate. The forages also had a higher concentration of linolenic acid and a lower concentration of linoleic acid than the concentrate.

	Grass silage	Concentrates	Grass
Dry Matter (DM a/ka)	178	858	179
Crude protein (a/kaDM)	170	138	13/
Ash (a/kaDM)	100	70	104
Asir (grkgDM) Oil (a/kaDM)	3/	10	30
DM Digostibility (DMD: g/kg)	5 7 620	17	700
	020	-	700
Fatty acid (FA) composition			
(g/100gFAmethyl esters)			
C 16:0	15.24	21.77	14.22
C 18:0	2.10	3.87	4.20
C 18:1	2.91	14.48	2.22
C 18:2	15.85	47.92	11.16
C 18:3	45.56	5.76	47.50
Saturated FA	21.43	27.04	21.68
Monounsaturated FA	4.40	16.54	5.13
Polyunsaturated FA	62.20	53.79	59.41

Table 1. Chemical composition and fatty acid profile of feedstuffs

Animals offered concentrates did not have any refusals, averaging a consumption of 2.57kg DM/day. Average DM intake (DMI) from silage was 4.10, 4.21 and 4.50kg/day for animals that were housed for 158, 118 and 59 days respectively, while DMI from grass was 8.52, 7.49 and 7.47kg/day for animals grazing for 40, 99 and 158 days respectively.

Animal performance data are summarised in Table 2. There was no significant difference between treatments for pre-slaughter weight. Cold carcass weight, average daily gain and peri-renal adipose tissue weight increased (quadratic P< 0.05) with duration of grazing prior to slaughter. Animals offered silage and concentrates for the duration of the experiment had the highest weight of perirenal fat, though not significantly higher than animals that were fed grass for the duration of the experiment.

	D	SED	P ¹			
	0	40	99	158		
Pre-slaughter weight (kg)	488	477	488	495	10.04	-
Cold carcass weight (kg)	258	247	248	258	5.50	Q 1
Average daily gain (kg)	0.959	0.876	0.900	0.996	0.0793	Q
Killout %	53.00	51.69	50.87	52.16	0.014	Q
KCF weight (kg)	6.06	4.50	4.62	5.15	0.611	Q

Table 2. Animal production

 ^{1}Q = quadratic effect of days at grass.

Fatty acid composition Intramuscular fat (Table 3)

Increasing the duration at pasture prior to slaughter resulted in a quadratic increase in the proportions of C_{14:0} and SFA (also linear), a linear increase in the proportions of C_{18:3} (n-3), C_{22:5}, C_{22:6}, CLA c_{18:1} trans 11, n-3 and total PUFA while there was no consistent pattern to the treatment effect on the proportions C_{16:0} and C_{18:1} (c_{15 9).}

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There was no effect of treatment on total fatty acid concentration in intramuscular lipid. Increasing the duration at pasture prior to slaughter resulted in a linear increase in the concentration of C_{18:3} (n-3), C_{20:5}, C_{22:5}, (also quadratic), CLA, C_{18:1} trans 11, n-3 and total PUFA, a linear decrease in the n-6:n-3 PUFA while there was no consistent pattern to the treatment effect on the concentration of C_{16:0}, total SFA or P:S.

	Days at grass					P ¹
	0	40	99	158		
Fatty acids						
(proportion×100)						
C14:0	2.08	2.53	2.31	2.09	0.141	**Q
C16:0	24.13	23.44	24.07	21.71	0.507	***
C18:0	16.94	17.51	16.93	17.12	0.591	NS
C18:1n9cis	35.70	33.15	34.22	33.60	0.944	*
C18:2n6 cis	2.64	2.52	2.35	2.49	0.178	NS
C18:3n3	1.03	1.14	1.02	1.29	0.088	*L
C20:4n6	0.12	0.14	0.11	0.17	0.024	0.07
C20:5n3	0.22	0.28	0.25	0.30	0.036	NS
C22:5n3	0.38	0.43	0.43	0.54	0.044	**L
C22:6n3	0.13	0.16	0.17	0.21	0.018	***L
CLA c9,t11	0.50	0.50	0.57	0.71	0.060	***L
C18:1n9trans	0.38	0.35	0.43	0.39	0.034	NS
C18:1n11trans	1.35	1.93	2.27	3.01	0.179	***L
Total SFA	45.40	45.84	45.49	43.23	0.772	**L,Q
Total MUFA	41.64	39.60	41.19	41.06	0.988	NS
Total PUFA	5.57	5.80	5.51	6.52	0.352	*L
n-6 fatty acids	3.25	3.20	2.97	3.31	0.231	NS
n-3 fatty acids	1.79	2.06	1.91	2.43	0.167	**L

Table 3. Relative and absolute fatty acid composition of intramuscular fat

Fatty acids						
(mg/100g muscle)						
C14:0	51.52	57.83	64.43	53.38	4.611	**Q
C16:0	596.6	550.8	668.9	542.5	46.02	*
C18:0	416.1	398.1	468.9	435.7	31.22	NS
C18:1n9cis	892.9	779.3	945.6	843.4	79.96	NS
C18:2n6	cis	62.11	63.74	59.37	58.99	3.321 NS
C18:3n3	19.59	25.38	30.92	34.43	1.857	***L
C20:4n6	3.48	3.37	3.54	3.22	0.310	NS
C20:5n3	5.59	5.51	6.41	7.67	0.502	***L
C22:5n3	10.14	9.38	10.61	12.74	0.738	***L,Q
C22:6n3	3.22	2.86	2.78	2.72	0.606	NS
CLA c9,t11	12.31	12.14	15.24	18.37	1.785	***L
C18:1n9trans	9.05	7.75	12.10	10.07	1.076	**
C18:1n11trans	32.46	44.91	60.15	76.64	4.538	***L
Total SFA	1117	1060	1262	1090	80.77	*
Total MUFA	1038	931.8	1135	1032	91.43	NS
Total PUFA	129.4	136.7	145.6	158.4	6.929	***L
P:S Ratio	0.12	0.14	0.12	0.15	0.009	*
n-6 fatty acids	77.25	79.34	76.83	78.61	3.871	NS
n-3 fatty acids	39.13	44.28	51.67	59.67	3.071	***L
n-6:n-3 Ratio	2.00	1.79	1.56	1.32	0.100	***L
Total fatty acids	2461	2329	2754	2515	177.5	NS

¹In this and subsequent tables, from experiment 1, L and Q are significant (P<0.05) linear and quadratic effects of days at grass, respectively; SFA = sum of saturated fatty acids, MUFA = sum of monounsaturated fatty acids, PUFA = sum of polyun-saturated fatty acids, P:S = ratio of PUFA to SFA

Adipose tissue (Table 4)

Of the important fatty acids, increasing the duration at pasture prior to slaughter resulted in a linear increase in the proportions of $C_{18:3}$ (n-3), CLA, C_{18:1} trans 11, C_{22:5}, total monounsaturated fatty acids (MUFA), PUFA and n-3 PUFA and a linear decrease in the proportions of C_{16:0} and SFA. There was no effect of treatment on total fatty acid concentration in adipose tissue. The treatment trends for individual fatty acid concentrations were similar to those for the proportions. In addition, increasing the duration at pasture prior to slaughter resulted in a linear increase in P:S and a linear decease in n-6:n-3 PUFA.

	Days at grass					Р
	0	40	99	158		
Fatty acids						
(proportion×100)						
C14:0	2.68	2.66	2.48	2.56	0.136	NS
C16:0	25.92	24.37	22.72	21.36	0.553	***L
C18:0	12.79	14.51	13.53	13.43	0.748	NS
C18:1n9cis	37.84	37.22	37.50	36.50	0.920	NS
C18:2n6 cis	1.15	1.09	1.09	1.06	0.050	NS
C18:3n3	0.52	0.54	0.71	0.88	0.041	***L
C20:4n6	0.04	0.04	0.05	0.04	0.006	NS
C22:5n3	0.06	0.06	0.10	0.10	0.021	*L
CLA c9,t11	0.66	0.77	1.11	1.64	0.14	***L
C18:1n9trans	0.46	0.51	0.86	0.91	0.140	***L
C18:1n11trans	1.34	1.78	2.91	4.10	0.390	***L
Total SFA	43.25	43.60	40.70	39.30	1.069	***L
Total MUFA	47.43	46.75	48.64	48.80	1.008	0.06L
Total PUFA	2.99	3.06	3.78	4.55	0.177	***L
n-6 fatty acids	1.65	1.57	1.67	1.72	0.063	NS
n-3 fatty acids	0.63	0.66	0.89	1.06	0.05	***L
Fatty acids						
(mg/g tissue)						
C14:0	15.90	16.89	14.89	15.98	1.096	NS
C16:0	153.2	154.6	136.5	132.9	6.032	***L
C18:0	75.44	91.93	81.89	83.66	5.357	*
C18:1n9cis	224.1	235.9	225.1	226.9	7.773	NS
C18:2n6 cis	6.79	6.89	6.51	6.59	0.381	NS
C18:3n3	3.09	3.38	4.28	5.44	0.293	***L
C20:4n6	0.26	0.27	0.27	0.26	0.035	NS
C22:5n3	0.38	0.41	0.61	0.64	0.128	0.09L
CLA c9,t11	3.98	4.85	6.68	10.23	0.925	***L

Table 4. Relative and absolute fatty acid composition of subcutaneous fat

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C18:1n9trans	2.79	3.13	5.23	5.62	0.926	***L
C18:1n11trans	8.10	11.27	17.67	25.67	2.642	***L
Total SFA	255.5	276.5	245.1	244.8	10.98	*
Total MUFA	281.2	296.2	292.1	303.5	10.57	NS
Total PUFA	17.80	19.27	22.67	28.32	1.414	***L
P:S Ratio	0.07	0.07	0.09	0.12	0.006	***L
n-6 fatty acids	9.80	9.94	10.01	10.72	0.535	NS
n-3 fatty acids	3.78	4.15	5.35	6.59	0.361	***L
n-6:n-3 Ratio	2.64	2.43	1.94	1.65	0.126	***L
Total Fatty Acids	592.1	633.5	601.2	622.4	19.74	NS

Meat Quality Variables

Table 5. Colour and composition of m. longissimus dorsi

	Days at grass					Р
	0	40	99	158		
Colour: muscle						
L value	36.24	34.87	34.89	34.73	0.612	L
a value	12.54	10.67	10.52	10.33	0.476	L
b value	8.03	6.90	6.65	6.41	0.309	L
Saturation	14.9	12.7	12.4	12.1	0.548	L
Hue	0.57	0.57	0.56	0.55	0.035	NS
Colour: fat						
L value	63.34	65.72	63.62	62.46	1.140	Q
a value	12.10	10.71	11.39	12.32	0.913	NS
b value	16.47	17.39	16.73	17.52	0.427	L
pН	5.36	5.40	5.44	5.42	0.069	NS
Drip Loss(%)	2.80	2.06	1.65	1.78	0.277	L
Composition (g/kg)						
Lipid	18.2	15.5	18.0	15.0	2.67	NS
Moisture	738.1	744.5	742.2	743.1	2.61	NS
Protein	232.3	233.7	232.3	234.9	0.21	NS
Ash	11.7	11.3	11.4	11.4	0.29	NS

Several meat quality variables are summarised in Table 5. There was no treatment effect on muscle pH measured at 48h post-slaughter. There was a linear increase in subcutaneous fat yellowness (b value) with increasing duration at grass before slaughter. Increasing the duration at pasture prior to slaughter resulted in a linear decrease in L (lightness), a (redness) and b (yellowness) values of muscle. These effects were reflected in a linear decrease in colour saturation. Increasing the duration at pasture prior to slaughter resulted in a linear decrease in drip loss. There was no treatment effect on muscle hue or chemical composition.

The data for shear force and cook loss are summarized in Table 6. Increasing the duration at pasture prior to slaughter resulted in a quadratic increase in shear force at all ageing times post-mortem and in cook loss after 7 days ageing post-mortem.

	Days at grass				SED	Р
	0	40	99	158		
Shear Force (N)						
Day 2	61.1	75.2	75.0	64.3	0.612	Q
Day 7	47.3	58.3	56.5	50.5	4.24	Q
Day 14	44.0	52.9	49.9	45.2	3.43	Q
Cook loss (%)						
Day 2	31.4	31.3	32.3	31.8	0.69	NS
Day 7	32.1	33.5	33.3	32.1	0.63	Q
Day 14	34.1	33.9	34.0	33.6	0.63	NS

Table 6. Shear force and cook loss data for m. longissimus dorsi

Sensory data are summarised in Table 7. The sensory attributes of tenderness, flavour, firmness, texture, chewiness or overall acceptability were not affected by pre-slaughter diet. For juiciness, there was no clear pattern of response to increasing duration at pasture prior to slaughter but muscle from animals that spent 40 days at pasture was rated more juicy than muscle from animals in the other treatment groups.



	Days at grass					Р
	0	40	99	158		
Tenderness ¹	5.54	5.40	5.38	5.52	0.309	NS
Juiciness	4.63	5.42	4.79	4.91	0.282	*
Flavour	4.12	4.22	4.17	4.28	0.130	NS
Firmness	5.19	5.38	5.33	5.40	0.195	NS
Texture	3.84	3.83	3.82	3.98	0.142	NS
Chewiness	3.36	3.42	3.49	3.40	0.187	NS
Acceptability	3.85	3.88	3.83	4.02	0.163	NS

Table 7. Sensory characteristics of m. longissimus dorsi

¹Tenderness 1-8; 1 = extremely tough, 8 = extremely tender. Moistness/Juiciness 1-8; 1 = extremely dry, 8 = extremely juicy. Overall flavour 1-6; 1 = extremely poor, 6 = extremely good. Overall firmness 1-8; 1 = extremely mushy, 8 = extremely firm. Overall texture 1-6; 1 = very poor, 6 = very good. Residual chewiness 1-6; 1 = not chewy, 6 = extremely chewy. Overall acceptability 1-6; 1 = not acceptable, 6 = extremely acceptable

Conclusions

- The beneficial effect of a grazed grass-based diet on the fatty acid composition of beef was confirmed.
- The scale of this beneficial effect is strongly dependent on the duration of grazing.
- The optimum concentration of beneficial fatty acids was not achieved suggesting that feeding management prior to grazing is important.
- Grazing influenced beef colour and drip-loss in a duration dependant manner.
- Animals finished at grass for 40 or 98 days produced meat that was tougher than that from animals finished on silage and concentrates or fed grass for the last 158 days.





4. EXPERIMENT 2:

The effects of fish oil inclusion and method of silage preservation on the fatty acid composition of intramuscular and subcutaneous adipose tissue of steers

Introduction

Ruminant milk fat and meat adipose tissue contain high concentrations of CLA produced by incomplete ruminal biohydrogenation of dietary linoleic acid and by tissue desaturation of vaccenic acid (C18:1 trans 11), also a product of incomplete biohydrogenation of dietary fatty acids. An increase in the concentration of CLA in muscle fat has been attained by dietary inclusion of plant oils rich in PUFA. Dietary inclusion of fish oil also increased the concentration of CLA in milk. Fishoil appears to alter the pattern of rumen biohydrogenation and promote the accumulation C18:1 trans11. The first objective of this study was to investigate the effect of increasing concentrations of dietary fish oil in the presence of linoleic acid (sunflower oil) in the diet of grass silage-fed steers on the fatty acid profile of M. Longissimus dorsi and subcutaneous adipose tissue.

Ruminant fat is a source of n-3 PUFA for humans, in particular of linolenic acid, which is the predominant fatty acid found in grass and grass silage. Wilting of silage prior to ensiling was shown to reduce the content of n-3 PUFA. The second objective of this experiment was to compare the effect of wilted and unwilted silage consumption on tissue fatty acid composition and to investigate the interactions between the type of silage and the concentration of fish oil in the diet. The third objective was to determine the impact of these nutritional modifications to the production system on the colour, and sensory characteristics of the resulting beef.

Materials and Methods Animal Management

Eighty Friesian steers (initial mean live weight of 566 kg, SD = 39.73 kg and approximately 24 months of age) were randomly assigned to one of eight dietary treatments in a randomised complete block design. They were housed in groups in a slatted floor shed and allowed individual access to their appropriate ration via electronic feeders. The dietary treatments were: unwilted grass silage and one of the four experimental concentrates described in Table 8 or wilted grass silage and one of the four experimental concentrates.

The silages were prepared from a primary growth of a predominantly perennial rye-grass sward. The swards were mowed and alternative rows picked up immediately with a precision chop harvester. Unwilted grass was rapidly ensiled in a covered silo with 3I Addsafer (48% formic acid, 16% ammonium tetraformate/I)/t grass. The remaining grass was conditioned during a mean wilting period of 32 hours, picked up as described above and ensiled without an additive in a covered silo. During the wilting period, there was no rain, the maximum temperature averaged 20.2°C, the minimum temperature averaged 5.7°C, there were on average, 13 hours of sunshine and relative humidity averaged 68.1%.

The grass had been conserved or 9 months when the experiment commenced. Animals were offered daily, 15 kg wilted silage or 30 kg unwilted silage in one feed and an average of 5 kg of the experimental concentrates distributed over 2 feeds. Allowances were adjusted based on increasing bodyweight and to maintain an estimated similar carcass growth rate among all treatments. The weight of all animals was monitored every three weeks.

The duration of the feeding period was 109 days on average. The animals were transported to a commercial slaughter facility and slaughtered within 1h of arrival. Slaughter and post-slaughter procedures were as described for experiment 1.

To estimate carcass composition, the cube-roll (6th to 10th rib joint) was collected at 24h post slaughter from animals offered either silage and concentrates that contained either 0 or 40 g fishoil/kg. This joint was separated into dissectable fat muscle and



bone and the weight of each component was used to calculate the joint composition. Chemical analysis was as described for experiment 1.

Ingredient (kg/tonne)	F0	F10	F20	F40
Barley	345	345	345	345
Sugar Beet Pulp	360	360	360	360
Soyabean Meal	140	140	140	140
Molasses	10	10	10	10
Min/vit	25	25	25	25
Oil:				
Sunflower	80	80	80	80
Fishoil	0	10	20	40
Lard	40	30	20	0
Total	1000	1000	1000	1000

Table 8. Formulation of Experimental Rations

NOTE: Min/Vit = 25000IU Vit E /t

Statistical analysis

Data were analysed using analysis of variance procedures and a model that had block, silage type, fishoil concentration and the interaction between silage type and fishoil concentration as main effects. The relationship between fishoil concentration and response variables was investigated using orthogonal polynomials. For cube roll composition, data were analysed using analysis of variance procedures and a model that had block, silage type, fishoil concentration and the interaction between silage type and fishoil concentration as main effects. For fatty acid data, means were calculated across duplicate samples and statistically analysed as described above.

Results

Animal performance

Feed composition and consumption data are reported in Table 9 and Table 10, respectively. Wilting of grass prior to ensiling increased the



DM concentration but decreased the concentration of total fatty acids and linolenic acid in silage. The linoleic acid and total fatty acid concentration was similar in all concentrates reflecting the constant inclusion of sunflower oil and the isoenergetic formulation strategy, respectively. Increasing the level of inclusion of fishoil increased the concentration of the longer chain PUFA in the concentrates. Animals offered wilted silage had higher silage and total DM consumption than animals offered unwilted silage.

	Unwilted silage	Wilted silage	F0	F10	F20	F40
DM (g/kg)	211	423	891	892	893	894
CP (g/kg DM)			139	143	137	144
Ash (g/kg DM)	88.5	96.6	58	60	64	64
DMD (g/kg)	753	784				
Oil (g/kg DM)	40.5	32.6	117	114	114	119
Fatty acid						
composition (g/kgDM)						
C 16:0	3.27	3.09	11.76	11.37	10.19	9.15
C 18:0	0.32	0.31	6.39	5.81	5.01	3.47
C 18:1	0.70	0.62	28.28	27.54	26.66	22.57
C 18:2	3.49	3.42	48.00	49.86	50.60	45.81
C 18:3	13.86	11.39	1.80	1.68	1.44	1.52
C 20:1	0.04	0.01	0.20	0.93	2.04	2.99
C 20:5	-	-	0.05	0.49	1.13	1.79
C 22:5	-	-	0.04	0.13	0.23	0.37
C 22:6	-	-	0.03	0.78	1.67	2.89
Saturated FA	4.57	4.20	19.65	18.99	17.24	20.80
Monounsaturated FA	1.30	1.46	29.40	29.92	30.89	37.80
Polyunsaturated FA	17.53	14.96	50.24	54.45	58.49	77.88
Total n-6 FA	3.68	3.57	48.60	51.68	54.02	69.81
Total n-3 FA	13.86	11.39	1.63	2.77	4.47	8.08
Total FA	26.30	24.12	101.6	106.1	109.0	103.7

Table 9. Chemical composition and fatty acid profile of feedstuffs

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Silage (S)	Unwilted				Wilted							
Fishoil (F, g/kg)	0	10	20	40	0	10	20	40	SED	S	F	S.F
Silage DM intake (kg)	6.22	6.31	6.14	6.26	6.64	6.59	6.51	6.62	0.106	***	NS	NS
Total DM intake (kg)	11.56	11.63	11.48	11.63	11.98	11.91	11.86	12.00	0.106	***	NS	NS

Table 10. Feed consumption

Animal production data are summarised in Table 11. There was no effect of silage type or fishoil inclusion in the concentrate and no interaction between these main effects for pre-slaughter weight or killing out percentage. There was a silage type by linear fishoil interaction for cold carcass weight. This is interpreted as a trend towards a linear decrease in carcass weight with increasing concentration of fishoil in the concentrate for animals offered unwilted silage but an opposite pattern for animals offered wilted silage. There was an interaction between silage type and fishoil inclusion in the concentrate for peri-renal adipose tissue weight. There was no clear pattern in the data, but the trend was for a quadratic decrease in animals offered the unwilted silage and a quadratic increase for animals offered the wilted silage.

Table 11. Ani	mal production	
Silage (S)	Unwilted	Wilt

Silage (S)	Unw	Unwilted				Wilted							
Fishoil (F, g/kg)	0	10	20	40	0	10	20	40	SED	S	F	S.F ¹	
Pre-slaughter weight (kg)	679	682	653	674	659	659	676	678	13.9	NS	NS	NS	
Cold carcass weight (kg)	345	344	337	338	333	328	341	344	6.9	NS	NS	L	
Killout (%)	50.8	50.5	50.8	50.1	50.5	49.9	50.4	50.7	0.69	NS	NS	NS	
Kidney/channel fat (kg)	14.5	10.0	12.0	12.6	12.5	14.4	12.7	13.0	1.15	NS	NS	*	

In this and subsequent tables ${}^{1}L$ = linear effect of fishoil inclusion in the concentrate.

Fatty acid composition: Intramuscular fat (Table 12)

Wilting of grass prior to ensiling decreased the proportion and concentration of C_{20:4}, C_{22:6}, total n-6 PUFA proportion and the n-6: n-3



PUFA ratio but increased the proportion and concentration of CLA. There was an interaction between silage type and fishoil inclusion in the concentrate for the total fatty acid concentration such that at the high level of inclusion, fatty acid concentration was increased for the unwilted silage-based ration but decreased for the wilted silage-based ration. A similar trend was observed for total MUFA and SFA. Fishoil inclusion linearly increased the concentration of CLA, C_{18:1} trans 11 and most long chain n-3 PUFA and linearly decreased the n-6:n-3 PUFA.

Silage (S)	Unwilted				Wilt	ed						
Fishoil (F, g/kg)	0	10	20	40	0	10	20	40	SED	S	\mathbf{F}^1	S.F
Fatty acids												
(proportionx100)												
C14:0	5.68	5.78	5.59	5.25	4.97	5.13	5.98	5.61	0.682	NS	NS	NS
C16:0	23.49	24.54	23.24	25.37	23.74	23.98	23.79	24.09	0.847	NS	NS	NS
C18:0	14.32	16.51	14.14	14.92	15.04	13.85	12.95	14.21	1.117	NS	NS	NS
C18:1n9cis	37.44	34.32	37.94	34.22	37.97	38.19	37.02	35.20	1.329	0.10	**L	*L
C18:2n6 cis	1.83	1.85	1.70	1.37	1.70	1.49	1.66	1.50	0.139	NS	**L	NS
C18:3n3	0.39	0.38	0.35	0.35	0.38	0.34	0.36	0.34	0.026	NS	NS	NS
C20:4n6	0.31	0.36	0.31	0.14	0.19	0.20	0.15	0.20	0.066	**	NS	*L
C20:5n3	0.06	0.09	0.08	0.07	0.04	0.06	0.06	0.10	0.015	NS	**L	*L
C22:5n3	0.14	0.16	0.14	0.13	0.13	0.14	0.12	0.17	0.020	NS	NS	NS
C22:6n3	0.02	0.02	0.03	0.03	0.01	0.01	0.02	0.03	0.006	*	**L	NS
CLA c9,t11	0.65	0.64	0.66	0.77	0.73	0.77	0.79	0.90	0.056	***	***L	NS
C18:1n9trans	0.23	0.24	0.20	0.30	0.29	0.28	0.29	0.33	0.047	*	NS	NS
C18:1n11trans	4.85	4.58	4.65	5.10	4.53	4.82	5.21	5.94	0.455	NS	**L	NS
Total SFA	45.77	49.17	4.522	47.87	46.01	45.17	44.93	46.21	1.567	0.07	NS	NS
Total MUFA	46.82	42.83	47.13	44.02	46.91	48.01	47.51	45.91	1.507	*	0.09	0.08
Total PUFA	3.66	3.81	3.54	3.20	3.42	3.27	3.42	3.57	0.230	NS	NS	*L
n-6 fatty acids	2.36	2.48	2.25	1.81	2.09	1.90	2.02	1.97	0.175	*	**L	*L
n-3 fatty acids	0.61	0.66	0.61	0.58	0.57	0.56	0.56	0.65	0.052	NS	NS	NS

Table 12. Relative and absolute fatty acid composition of intramuscular fat

Fatty acids												
(mg/100g muscle)												
C14:0	362.2	286.7	336.8	387.1	352.8	368.5	401.2	337.5	54.54	NS	NS	NS
C16:0	1517	1249	1364	1987	1701	1697	1595	1524	206.9	NS	NS	*L
C18:0	913.9	830.8	811.3	1161	1070	945.0	868.3	907.1	122.1	NS	NS	NS
C18:1n9cis	2488	1736	2325	2694	2684	2780	2494	2252	335.1	NS	NS	*L
C18:2n6 cis	113.3	90.71	102.1	103.2	115.5	105.5	111.2	90.73	9.921	NS	0.053	NS
C18:3n3	24.37	18.93	21.15	26.76	26.28	24.13	23.80	21.17	2.601	NS	NS	*L
C20:4n6	18.52	17.41	17.09	10.17	12.67	13.58	10.09	11.40	3.498	*	NS	NS
C20:5n3	3.47	4.11	4.46	5.17	2.98	4.04	3.66	5.35	0.748	NS	***L	NS
C22:5n3	8.94	7.75	8.51	9.36	8.45	9.40	8.07	9.96	1.061	NS	NS	NS
C22:6n3	1.29	1.01	1.68	2.02	0.87	0.95	1.02	1.77	0.341	*	***L	NS
CLA c9,t11	42.52	32.34	40.91	60.24	50.50	54.32	53.15	56.84	7.010	**	**L	NS
C18:1n9trans	15.18	11.66	11.60	24.05	20.04	19.89	19.92	21.13	3.773	*	*L,Q	NS
C18:1n11trans	309.9	230.8	287.6	390.3	309.8	334.1	349.0	377.9	41.83	0.074	**L	NS
Total SFA	2941	2484	2647	3716	3283	3168	3013	2914	357.6	NS	NS	*L
Total MUFA	3106	2166	2895	3460	3310	3487	3194	2928	438.9	NS	NS	*L
Total PUFA	229.5	186.5	212.9	244.0	234.0	229.5	228.8	218.3	21.09	NS	NS	NS
P:S Ratio	0.08	0.08	0.08	0.07	0.07	0.07	0.08	0.08	0.006	NS	NS	NS
n-6 fatty acids	146.5	120.7	133.7	136.8	141.9	133.5	135.1	119.1	12.24	NS	NS	NS
n-3 fatty acids	38.50	32.10	36.28	44.08	38.84	38.96	37.50	38.66	3.459	NS	NS	NS
n-6:n-3 Ratio	3.86	3.82	3.69	3.12	3.68	3.41	3.63	3.10	0.178	0.063	***L	NS
Total fatty acids	6521	5045	6009	7802	7078	7131	6713	6331	809.3	NS	NS	*L

In this and subsequent tables, Q = quadratic effect of fishoil concentration in the concentrate.

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Silage (S)	Unwilted				Wilt	ed						
Fishoil (F, g/kg)	0	10	20	40	0	10	20	40	SED	S	F	S.F
Fatty acids												
(proportion×100)												
C14:0	3.74	3.88	3.90	3.91	3.86	3.36	3.87	3.69	0.305	NS	NS	NS
C16:0	21.77	21.20	21.30	20.84	20.64	20.92	21.09	21.08	1.056	NS	NS	NS
C18:0	9.51	10.41	8.95	8.83	8.67	8.79	8.89	8.61	0.904	NS	NS	NS
C18:1n9cis	40.70	40.43	41.14	39.80	41.63	42.19	40.13	38.82	1.896	NS	NS	NS
C18:2n6 cis	1.24	1.10	1.14	1.11	1.43	1.14	1.24	1.08	0.076	0.06	***L	NS
C18:3n3	0.26	0.23	0.21	0.25	0.29	0.23	0.26	0.22	0.025	NS	*	NS
C20:4n6	0.05	0.05	0.05	0.05	0.04	0.04	0.05	0.06	0.015	NS	NS	NS
C22:5n3	0.06	0.04	0.04	0.04	0.03	0.05	0.04	0.05	0.011	NS	NS	*L
CLA c9,t11	1.01	1.10	1.07	1.33	1.27	1.34	1.35	1.66	0.136	***	***L	NS
C18:1n9trans	0.37	0.51	0.64	0.86	0.57	0.32	0.50	0.97	0.297	NS	*L	NS
C18:1n11trans	5.42	5.36	4.88	5.35	4.20	4.39	5.12	6.27	0.791	NS	0.06L	NS
Total SFA	37.72	37.88	36.78	36.21	35.71	35.54	36.43	36.01	1.900	NS	NS	NS
Total MUFA	54.16	53.59	54.86	54.56	54.94	55.51	54.46	54.18	1.906	NS	NS	NS
Total PUFA	2.93	2.73	2.76	3.12	3.41	3.06	3.27	3.38	0.244	**	NS	NS
n-6 fatty acids	1.53	1.32	1.39	1.43	1.77	1.39	1.56	1.38	0.116	NS	*L	NS
n-3 fatty acids	0.33	0.27	0.27	0.32	0.32	0.28	0.31	0.28	0.031	NS	NS	NS
Fatty acids												
(mg/g tissue)												
C14:0	20.60	20.85	19.82	19.96	19.11	18.86	18.96	19.28	2.051	NS	NS	NS
C16:0	119.3	113.6	108.0	105.6	102.0	115.3	104.1	109.7	7.736	NS	NS	NS
C18:0	52.24	55.18	45.20	45.26	42.58	48.36	43.79	44.80	4.983	0.07	NS	NS
C18:1n9cis	221.3	217.3	209.3	202.7	208.3	230.5	197.8	202.2	16.23	NS	0.06L	NS
C18:2n6 cis	6.79	5.96	5.84	5.61	7.13	6.23	6.13	5.63	0.512	NS	***L	NS
C18:3n3	1.44	1.23	1.10	1.30	1.45	1.23	1.29	1.12	0.145	NS	*L	NS
C20:4n6	0.26	0.25	0.23	0.24	0.19	0.20	0.26	0.29	0.072	NS	NS	NS
C22:5n3	0.33	0.20	0.21	0.22	0.14	0.24	0.19	0.25	0.060	NS	NS	*L
CLA c9,t11	5.47	6.00	5.48	6.78	6.37	7.34	6.62	8.67	0.756	***	**L	NS
C18:1n9trans	1.70	0.90	2.93	3.43	1073	1.32	2.42	1046	1.430	NS	NS	NS
C18:1n11trans	30.72	28.40	24.68	27.45	20.54	23.97	24.98	32.74	4.672	NS	NS	NS

Table 13. Relative and absolute fatty acid composition of subcutaneous fat

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Silage (S)	Unwilted				Wilted							
Fishoil (F, g/kg)	0	10	20	40	0	10	20	40	SED	S	F	S.F
Total SFA	206.7	202.5	186.4	184.1	176.3	196.1	179.6	187.4	13.50	NS	NS	NS
Total MUFA	295.9	287.7	279.3	278.0	274.5	303.7	268.1	282.6	16.54	NS	NS	NS
Total PUFA	16.00	14.78	14.11	15.90	17.07	16.72	16.13	17.57	1.455	*	NS	NS
P:S Ratio	0.08	0.08	0.08	0.09	0.10	0.09	0.09	0.09	0.010	*	NS	NS
n-6 fatty acids	8.38	7.11	7.10	7.27	8.86	7.55	7.70	7.17	0.706	NS	*L	NS
n-3 fatty acids	1.82	1.47	1.37	1.62	1.62	1.51	1.55	1.46	0.175	NS	NS	NS
n-6:n-3 Ratio	4.89	4.94	5.30	4.61	5.58	5.26	4.99	5.04	0.477	NS	NS	NS
Total fatty acids	546.9	536.3	508.1	509.1	497.3	549.0	492.9	521.2	25.24	NS	NS	NS

Meat Quality

Muscle pH and colour data are summarised in Table 14. There was no effect of silage type or fishoil inclusion in the concentrate and no interaction between these main effects on the ultimate pH, L (lightness), a (redness) and b (yellowness) values of longissimus dorsi muscle. Similarly, there was no treatment effect on muscle saturation. There was no effect of silage type on muscle hue. Muscle hue was increased by an increase in the concentration of fishoil in the concentrate but the pattern was complex with both linear and cubic components being significant.

Silage (S)	Unw	Unwilted				Wilted						
Fishoil (F, g/kg)	0	10	20	40	0	10	20	40	SED	S	F ¹	S.F
pН	5.51	5.58	5.59	5.57	5.58	5.54	5.58	5.55	0.027	NS	NS	NS
"L"	33.8	33.7	33.0	34.5	33.1	33.9	34.3	33.7	0.71	NS	NS	NS
"a"	14.4	13.4	13.2	13.7	13.1	13.6	13.2	12.9	0.64	NS	NS	NS
"b"	7.9	7.5	7.1	7.8	7.1	7.5	7.3	7.4	0.37	NS	NS	NS
Saturation	16.5	15.3	15.0	15.8	15.0	15.5	15.1	14.9	0.73	NS	NS	NS
Hue	0.50	0.51	0.49	0.51	0.50	0.50	0.51	0.52	0.008	NS	L,C	NS

Table	14.	pН	and	colour	of	m.	longissimus	dorsi

In this and subsequent tables C = cubic effect of fishoil concentration in the concentrate.

Additional meat quality data are summarised in Table 15. Fishoil inclusion in the concentrate tended to increase drip loss at low levels of inclusion and to decrease drip loss at high levels of inclusion. The response pattern had significant linear and cubic terms. Shear force was linearly decreased with increasing level of fishoil inclusion after 2 and 7 days ageing post-mortem. For 14 day-aged muscle, wilting of grass prior to slaughter resulted in a decrease in shear force in muscle. For cook loss from muscle, there was a complex pattern of response to increasing the level of fishoil inclusion in the concentrate but the absolute effects were small.

Silage (S)	Unwilted				Wilted							
Fishoil (F, g/kg)	0	10	20	40	0	10	20	40	SED	S	F	S.F
Drip loss (%)	0.78	1.41	0.69	0.43	0.71	0.82	0.73	0.68	0.238	NS	L,C	NS
Shear force (N)												
Day 2	46.4	43.6	47.5	37.1	51.6	42.4	41.9	40.4	7.05	NS	L	NS
Day 7	40.7	40.2	40.1	35.3	36.1	39.8	38.4	31.5	3.59	NS	L	NS
Day 14	35.5	32.4	41.2	31.5	29.2	31.4	29.3	31.5	4.01	*	NS	NS
Cook loss (%)												
Day 2	30.5	29.8	31.8	27.4	27.8	29.6	28.6	30.1	1.19	NS	NS	L,C
Day 7	30.8	32.0	30.5	29.9	29.4	29.5	30.3	30.0	0.78	*	NS	L
Day 14	30.9	30.5	31.1	28.8	28.9	31.9	28.6	31.4	1.04	NS	NS	L,C

Table 15. Drip loss, shear force and cook loss of m. longissimus dorsi

Rib composition is shown in Table 16. There was no evidence that wilting of grass prior to ensiling or fishoil inclusion in the concentrate influenced the chemical composition of an indicator (of carcass composition) joint of the carcass

Silage (S)	Unwilt	ed	Wilted	Wilted				
Fishoil (F, g/kg)	0	40	0	40	SED	S	F	S.F
Fat	235	249	232	216	16.5	NS	NS	NS
Muscle	555	546	554	573	13.8	NS	NS	NS
Bone	210	205	215	211	5.4	NS	NS	NS

Table 16. Composition	n of rib	joint	(g/kg)
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Sensory data are summarised in Table 17. There was a tendency for muscle from cattle fed the high fishoil concentrate to be more tender while muscle from cattle fed wilted silage had higher scores for flavour and overall acceptability.

Silage (S)	Unwilted			Wilted								
Fishoil (F, g/kg)	0	10	20	40	0	10	20	40	SED	S	F	S.F
Tenderness ¹	5.73	5.65	5.68	6.09	6.24	5.79	5.75	6.16	0.362	NS	Q+	NS
Juiciness	5.08	5.53	5.43	5.38	5.69	5.01	5.33	5.70	0.368	NS	NS	Q+
Flavour	4.36	4.54	4.54	4.37	4.65	4.53	4.53	4.74	0.149	*	NS	Q
Firmness	5.45	5.57	5.53	5.16	5.36	5.45	5.43	5.23	0.229	NS	NS	NS
Texture	4.14	4.18	4.16	4.04	4.38	4.17	4.09	4.49	0.209	NS	NS	NS
Chewiness	3.31	3.23	3.51	3.31	3.00	3.29	3.25	3.16	0.242	NS	NS	NS
Acceptability	4.11	4.25	4.21	3.98	4.46	4.28	4.20	4.46	0.211	*	NS	Q+

Table 17.	Sensorv	characteristics (of m.	lonaissimus	dorsi

¹Tenderness 1-8; 1 = extremely tough, 8 = extremely tender Moistness/Juiciness 1-8; 1 = extremely dry, 8 = extremely juicy Overall flavour 1-6; 1 = extremely poor, 6 = extremely good Overall firmness 1-8; 1 = extremely mushy, 8 = extremely firm Overall texture 1-6; 1 = very poor, 6 = very good Residual chewiness 1-6; 1 = not chewy, 6 = extremely chewy Overall acceptability 1-6; 1 = not acceptable, 6 = extremely acceptable

Conclusions

- Fish oil supplementation enhanced the concentration in beef, of fatty acids that are beneficial to human health.
- The linear response to increasing level of fish oil consumption indicates scope to further enhance the concentrations of beneficial fatty acids in beef.
- Wiliting of grass prior to ensiling did not impact negatively on the overall content of n-3 PUFA in muscle, but it increased the concentration of CLA.
- Dietary inclusion of fish oil or wilting of grass prior to ensiling did not affect muscle appearance.
- Fish oil seemed to increase tenderness but only at the high level of inclusion. This merits further study.
- There was some evidence that wilting of grass prior to ensiling enhanced meat tenderness. This needs to be confirmed.



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