

MEAT QUALITY

Effects of feeding level, feeding method, breed type and duration of finishing on muscle chemical composition of steers and relationships between carcass and muscle compositional traits

The effects of feeding level and method of feeding (feeds offered as discrete ingredients or as a total mixed ration (TMR)) have recently been studied in beef cattle but muscle chemical composition was not reported. A knowledge of beef muscle chemical composition is essential in order to assess its nutritive value and its role in the human diet. With increasing consumer concerns over the consequences of high animal fat consumption, it is important that the fat content of beef be acceptably low. However, if it is too low organoleptic properties, which are associated with intramuscular or marbling fat content, may be impaired.

It has for long been accepted that there is a general parallel between the chemical composition of carcass tissues and the proportions of separable tissues in the carcass but the relationships may vary with feeding level, breed type or slaughter end point. As it is not always practical to slaughter cattle at the same carcass weight, it is best to use serial slaughter when studying carcass and tissue growth of different breed types.

As measurement of both carcass composition and muscle chemical composition are costly and involve a time delay after slaughter, it would be of great practical benefit if important carcass and tissue compositional traits could be predicted from routinely recorded carcass traits such as weight and fat score. Relationships between compositional traits and carcass weight would likely only apply within breed because of the differences between breeds in the relative growth rates of carcass tissues. Relationships between carcass fat score and muscle lipid concentration might hold across breeds however, because of the relatively minor differences between breeds in fat depot growth coefficients.

The objectives of this study were (i) to determine the effects of feeding level (supplementary concentrates with grass silage), feeding method (feeds offered separately or as a total mixed ration (TMR)), breed type (Friesian or Charolais x Friesian) and duration of finishing period on *m. longissimus* chemical composition of steers, and (ii) to describe the relationships between certain carcass traits and carcass and muscle composition.

In this experiment 65 Friesian and 52 Charolais x Friesian finishing steers were assigned on live weight within breed type to a pre-experimental slaughter group and 12 finishing groups. The pre-experimental group was slaughtered the following day. The 12 finishing groups were assigned in a 6 x 2 factorial design to 6 feeding treatments x 2 durations of finishing. The 6 feeding treatments were:

1. Grass silage (SO) only offered *ad libitum*.
2. SO plus a low level of supplementary concentrates offered separately (LS).
3. SO plus a low level of supplementary concentrates offered as a TMR (LM).
4. SO plus a high level of supplementary concentrates offered separately (HS).
5. SO plus a high level of supplementary concentrates offered as a TMR (HM).
6. Concentrates offered *ad libitum* with restricted silage (AL).

The two durations of finishing were 105 (S) and 175 (L) days.

Mean low and high concentrate levels were 0.415 and 0.732 of daily dry matter (DM) intake, respectively. The concentrate composition was (kg/t): rolled barley 870, soya bean meal 67.5, molasses 47.5 and mineral/vitamin premix 15. The DM concentration of the silage was 198 g/kg and the analysis (g/kg) of the DM was crude protein 143 and *in vitro* digestibility 698. The pH was 3.9.

After slaughter, cold carcass weight was estimated as 0.98 of hot carcass weight. Carcasses were graded for conformation and fatness, and perinephric plus retroperitoneal fat weight was

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recorded. The right side of each carcass was quartered at the 10th rib and fat depth was measured. The ribs joint (ribs 6 to 10) was removed and separated into subcutaneous fat, intermuscular fat, *m. longissimus*, other muscle and bone (including *ligamentum nuchae*). A sample of *m. longissimus* was frozen and later chemically analysed for moisture, protein and lipid concentrations. Lipid and moisture were determined using an integrated microwave moisture and methylene chloride fat extraction method on a CEM moisture/solids analyser. Protein was determined using a LECO protein analyser.

The data were statistically analysed using the general least squares linear model procedures of the Statistical Analysis Systems Institute (SAS, 1996). The final model had terms for block, feeding treatment, breed and duration of finishing. The effects of the feeding treatments were partitioned, using *a priori* contrasts, into the linear, quadratic and cubic effects of concentrate level and the effect of feeding method (separate feeds or TMR). As the cubic effect of concentrate level was not significant it is not reported. Ribs joint tissue proportions and muscle chemical constituents were regressed on carcass weight and carcass fat score both within breed and overall. Muscle moisture and protein concentrations were regressed on muscle lipid concentrations. The data are presented as means for the pre-experimental slaughter group (\pm s.d.), the six feeding treatments (\pm s.e.), the two breed types and the two finishing periods with the significance levels indicated. The regressions, which include the pre-experimental slaughter group data are shown for the overall data set and for the two breed types separately.

Selected slaughter and ribs joint compositional traits, together with *m. longissimus* chemical composition are shown in Table 67. Starting live weight was similar for the pre-experimental slaughter group, the six feeding treatment groups and the two finishing period groups, but the Charolais crosses were heavier ($P < 0.001$) than the Friesians. At slaughter, the L group was heavier ($P < 0.001$) than the S group as intended. There were significant ($P < 0.001$) linear and quadratic effects of concentrate level on slaughter weight but there were no significant effects of feeding method or breed type. There was a significant ($P < 0.001$) linear effect of concentrate level, and significant ($P < 0.001$) breed type and duration of finishing effects on kill-out proportion. Both the linear and quadratic effects of concentrate level were significant ($P < 0.001$) for carcass weight, which was greater ($P < 0.001$) for Charolais crosses than for Friesians, and for L than S. There was no effect of feeding method on carcass weight.

Carcass fat class, perinephric plus retroperitoneal fat weight, and fat depth all increased ($P < 0.001$) linearly and quadratically with increasing concentrate level. The breed type ($P < 0.07$) and duration of finishing ($P < 0.06$) effects on carcass fat class were close to significance but there was no method of feeding effect. There were significant ($P < 0.01$) linear and quadratic effects of concentrate level on ribs joint total fat and bone proportions, and a significant ($P < 0.05$) linear effect on total muscle proportion. The breed type effect was significant ($P < 0.001$) for all three ribs joint components, and duration of finishing was significant ($P < 0.001$) for ribs joint total fat and muscle proportions. *M. longissimus* moisture, protein and lipid concentrations were all significantly linearly ($P < 0.001$) and quadratically ($P < 0.05$) related to concentrate level, and there were significant ($P < 0.001$) breed type and duration of finishing effects for moisture and lipid concentrations but there was no effect of feeding method.

Regressions on carcass weight, carcass fat class and *m. longissimus* lipid concentration both for the breed types separately and overall are shown in Table 68. While relationships were generally highly significant, the R^2 values were moderate to low. For the overall data set, carcass weight was moderately predictive of kill-out proportion, carcass conformation class, carcass fat class, and perinephric plus retroperitoneal fat weight. Fatness traits were more closely related to carcass weight for Friesians than for Charolais crosses but the opposite was so for kill-out proportion. Carcass weight was generally a better predictor of ribs joint composition for Friesians than for Charolais crosses, and *m. longissimus* composition,

particularly moisture and lipid concentrations, were more closely related to carcass weight for Friesians than for Charolais crosses.

Carcass fat class was a better predictor of perinephric plus retroperitoneal fat weight and ribs joint composition for Friesians than for Charolais crosses, and was also a better predictor of *m. longissimus* moisture concentration for Friesians. However, it was a better predictor of *m. longissimus* lipid concentration for Charolais crosses. *M. longissimus* moisture concentration was closely and negatively related to lipid concentration, with no differences between breed types, but *m. longissimus* protein concentration was poorly related to lipid concentration.

The similarity in kill-out proportion for the pre-experimental slaughter and silage only groups would be expected from the low live weight gains of the latter during finishing as kill-out is low when feeding level and growth rate are low. However, all measures of fatness were higher for the silage only group than for the pre-experimental slaughter group indicating that even at their low rate of gain there was still some fat deposition.

As the Charolais crosses were heavier than the Friesians at the start the absence of a difference in slaughter weight was unexpected as Charolais crosses usually grow faster than Friesians. Despite their similar slaughter weight however, the Charolais crosses had heavier carcasses due to their higher kill-out proportion.

There were large effects of concentrate level on moisture and lipid concentrations of *m. longissimus* but the effects on protein concentration were small. In fact, other than for the silage only group, there were no differences between the feeding treatments in *m. longissimus* protein concentration. Neither was there any effect of duration of finishing on protein concentration and the breed effect ($P < 0.08$) failed to reach formal significance. This suggests that muscle protein concentration is relatively little affected by production factors such as nutrition, breed type and slaughter weight. Generally, the changes in *m. longissimus* chemical composition support the contention that after a certain live weight, the protein concentration muscle remains relatively stable whereas the lipid concentration increases and the moisture concentration decreases with increasing weight.

The rather poor relationship between carcass weight and kill-out proportion, even within breed type, may have been due to differences in gut contents resulting in inaccuracies in the measurement of slaughter weight, one of the components of kill-out proportion. Relationships involving carcass conformation and fat classes were probably influenced by the relatively narrow range of these classes in the data set. The overwhelming majority of carcasses fell into two conformation classes (O and R, numerical values 2 and 3), and into the fat classes 3 and 4L (numerical values 3.0 and 3.7). When one variable in a relationship covers a wide range of values while the other is confined to a narrow range, a good fit is unlikely because a single value of one covers a range of values of the other.

Since the relationship between carcass weight and composition differs with breed the different regression coefficients for ribs joint tissue proportions on carcass weight for the two breed types would be expected. As the relationships of fat and bone proportions with carcass weight were better than for muscle proportion it suggests that when predicting ribs joint composition and ultimately carcass composition from carcass weight, it would be preferable to predict fat and bone from the regression equations and estimate muscle proportion by difference. The better relationships between carcass weight and *m. longissimus* moisture and lipid concentrations within breeds than overall is due to the fact that relationships between weight and tissue composition differ with breed type.

While most of the relationships presented were highly significant only a few are likely to be useful in predicting carcass grades or compositional traits from carcass weight or carcass fat class. For Friesians, carcass weight predicted carcass fat class, ribs joint fat and bone

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proportions, and *m. longissimus* moisture and lipid concentrations with reasonable accuracy. For Charolais crosses, the only trait predicted from carcass weight with reasonable accuracy was kill-out proportion. The probable reason for the better relationships between carcass weight and fat variables in Friesians was their greater range in fatness.

The close relationship between *m. longissimus* lipid and moisture concentrations both within breeds and overall, suggests that for practical purposes, measurement of one or other of these should be sufficient to estimate muscle chemical composition. As ash concentration is low and relatively stable, and protein concentration is also relatively stable, and as all constituents must sum to 1000 g/kg, measurement of either moisture or lipid alone would permit estimation of the others.

It is concluded that at similar slaughter weights, Charolais crosses had a higher kill-out proportion and carcass weight than Friesians. They also had better carcass conformation, had higher muscle and lower fat and bone proportions in the ribs joint, and had more moisture and less lipid in *m. longissimus*. *M. longissimus* moisture concentration decreased and lipid concentration increased with increasing concentrate level and length of finishing period. Relationships of carcass weight and carcass fat class with ribs joint compositional traits were better for Friesians than for Charolais crosses. *M. longissimus* moisture concentration was closely related to lipid concentration.

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Table 67: Effects of feeding treatment, breed type and duration of finishing on slaughter traits, ribs joint composition and *m. longissimus* chemical composition of finishing steers

	Feeding treatment (F)							Breed (B)		Duration of finishing (D)			Significance				
	PS (\pm s.d.)	SO	LS	LM	HS	HM	AL	FR	CH	S	L	s.e. ¹	F	B	D	L ²	Q ³
Start weight (kg)	477 (37.1)	476	476	476	476	476	476	466	486	475	478	6.3	NS	***	NS	NS	NS
Slaughter weight (kg)	477 (37.1)	506	601	605	630	626	641	601	602	577	626	9.1	***	NS	***	***	***
Kill-out (g/kg)	506 (15.3)	510	520	523	529	526	536	504	543	515	532	4.2	***	***	***	***	NS
Carcass weight (kg)	241.4 (24.5)	258.5	312.5	316.5	332.5	328.8	343.7	303.2	327.6	297.4	333.4	4.93	***	***	***	***	***
Conformation ⁴	2.04 (0.320)	2.02	2.25	2.34	2.47	2.43	2.78	1.97	2.80	2.42	2.35	0.088	***	***	NS	***	NS
Fat score ⁵	1.92 (0.419)	2.78	3.51	3.54	3.62	3.59	3.70	3.37	3.54	3.37	3.54	0.106	***	<0.07	<0.06	***	***
Perinephric + retroperitoneal fat (kg)	3.9 (3.05)	6.4	12.1	12.3	12.0	12.4	12.5	12.7	9.9	9.5	13.5	0.83	***	***	***	***	***
Fat depth (mm)	2.1 (2.11)	4.9	9.1	9.7	10.4	10.6	11.3	8.9	9.8	8.3	10.3	0.61	***	<0.06	***	***	***
<u>Ribs composition (g/kg)</u>																	
Subcutaneous fat	41 (12.2)	33	56	55	61	64	63	52	59	54	57	3.7	***	*	NS	***	***
Intermuscular fat	108 (28.0)	129	153	160	176	179	182	180	146	146	181	7.6	***	***	***	**	<0.08
Total fat	150 (31.0)	163	209	216	237	242	245	232	205	199	238	9.1	***	***	***	***	**
<i>M. longissimus</i>	181 (15.9)	208	192	198	196	197	202	185	212	204	194	4.9	NS	***	*	NS	*
Other muscle	449 (24.6)	401	393	386	376	372	369	375	391	39.5	371	6.6	**	**	***	*	NS
Total muscle	630 (29.3)	609	584	584	572	569	571	560	603	599	565	8.0	**	***	***	*	NS
Bone	221 (16.1)	228	207	201	190	189	184	208	192	202	198	4.2	***	***	NS	***	**
<u><i>M. longissimus</i> Composition (g/kg)</u>																	
Moisture	757 (8.6)	752	731	727	724	720	721	724	734	738	721	3.2	***	***	***	***	***
Protein	226 (4.7)	220	228	227	227	229	229	225	228	226	227	1.6	**	<0.08	NS	**	*
Lipid	10 (9.7)	19	33	39	41	43	42	42	30	28	44	3.9	***	***	***	**	**

¹For Feeding treatment, n = 18; ²Linear effect of concentrate level; ³Quadratic effect of concentrate level; ⁴EU Beef Carcass Classification Scheme – scale 1 = P (poorest) to 5 = E (best); ⁵EU Beef Carcass Classification Scheme – scale 1 (leanest) to 5 (fattest). There was no significant effect of method of feeding and no significant Feeding treatment x Duration of finishing or Breed type x Duration of finishing interactions. PS = Pre-experimental slaughter group.

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Table 68: Regressions ($y = a + bX$) of carcass traits, ribs joint tissue proportions and *m. longissimus* chemical constituents on carcass weight, carcass fat score and *m. longissimus* lipid proportion

	All data						Friesians						Charolais x Friesians					
	Intercept (a)	s.e. (a)	b	s.e. (b)	Adj R^2	Sig.	Intercept (a)	s.e. (a)	b	s.e. (b)	Adj R^2	Sig.	Intercept (a)	s.e. (a)	b	s.e. (b)	Adj R^2	Sig.
<u>X = Carcass weight (kg)</u>																		
Kill-out (g/kg)	407	14.0	0.37	0.045	0.36	***	434	14.60	0.23	0.049	0.26	***	433	16.4	0.33	0.051	0.45	***
Conformation	0.32	0.325	0.0064	0.0010	0.24	***	0.95	0.225	0.0034	0.0007	0.23	***	0.93	0.474	0.006	0.0015	0.21	***
Fat score	0.47	0.355	0.0093	0.0011	0.36	***	-1.11	0.467	0.015	0.0016	0.58	***	1.92	0.501	0.005	0.0016	0.14	***
Perinephric + retroperitoneal fat (kg)	-6.7	2.71	0.057	0.0087	0.27	***	-15.4	3.73	0.092	0.0124	0.46	***	-5.7	2.95	0.048	0.0091	0.34	***
Total fat (g/kg)	18.7	29.30	0.64	0.094	0.28	***	-76.7	35.97	1.02	0.119	0.53	***	39.8	40.35	0.51	0.125	0.24	***
Total muscle (g/kg)	660	28.2	-0.26	0.091	0.06	**	721	31.9	-0.53	0.106	0.27	***	685	38.8	-0.26	0.120	0.07	*
Total bone (g/kg)	322	11.3	-0.39	0.036	0.49	***	356	16.1	-0.49	0.054	0.56	***	275	14.8	-0.25	0.046	0.37	***
Moisture (g/kg)	812	10.9	-0.26	0.035	0.32	***	844	13.9	-0.39	0.046	0.53	***	811	13.6	-0.23	0.042	0.37	***
Protein (g/kg)	213	4.6	0.04	0.015	0.06	**	216	7.4	0.03	0.025	0.008	NS	210	5.9	0.05	0.018	0.13	**
Lipid (g/kg)	-33	11.9	0.22	0.038	0.22	***	-67	16.2	0.36	0.054	0.40	***	-29	14.6	0.18	0.045	0.23	***
<u>X = Fat score</u>																		
Perinephric + retroperitoneal fat (kg)	-0.06	1.997	3.30	0.589	0.21	***	-1.20	2.505	4.09	0.724	0.33	***	0.55	3.041	2.60	0.875	0.13	**
Total fat (g/kg)	60	19.7	46.8	5.81	0.36	***	62	21.9	50.6	6.60	0.48	***	36	34.5	48.8	9.93	0.31	***
Total muscle (g/kg)	667	19.0	-25.7	5.62	0.15	***	654	18.4	-27.9	5.53	0.28	***	723	32.1	-35.2	9.25	0.21	***
Total bone (g/kg)	273	9.1	-21.1	2.68	0.34	***	284	10.5	-22.7	3.17	0.44	***	241	15.6	-13.6	4.48	0.14	**
Moisture (g/kg)	780	8.2	-14.7	2.43	0.24	***	783	9.3	-17.4	2.80	0.37	***	781	14.2	-13.0	4.10	0.15	**
Protein (g/kg)	220	3.32	1.9	0.978	0.02	*	217	4.7	2.5	1.26	0.04	*	226	5.7	0.30	1.649	0.12	NS
Lipid (g/kg)	-6	8.76	12.4	2.58	0.16	***	-8	10.6	14.6	3.18	0.24	***	-18	8.2	0.23	0.039	0.40	***
<u>X = Lipid</u>																		
Moisture	763	1.33	-0.92	0.033	0.87	***	763	1.92	-0.91	0.042	0.88	***	765	1.93	-0.95	0.057	0.85	***
Protein	229	1.29	-0.08	0.032	0.04	*	230	1.92	-0.104	0.043	0.07	**	227	1.81	-0.03	0.053	0.02	NS

A three-dimensional study of bovine hoof growth using stable isotope analysis

Incremental tissues such as hair, hoof and teeth can reveal information about the diet assimilated at the time of tissue growth as they are not turned over once the tissue is laid down or mineralised. Therefore, such tissues can be used to gain information on seasonal feeding and movement behaviour. Our previous research on bovine hooves showed that it is possible to gather retrospective information on changes in diet by applying a superficial drilling method to the bovine hoof wall. However, unlike hair, hooves have three-dimensional structures. Understanding hoof growth in three dimensions would help to avoid the introduction of artefacts (e.g. due to mixing of tissues of different ages) and lead to suggestions improved for sampling strategies to maximise the information gained. To develop and advance this method, it is necessary to understand the mechanism by which the bovine hoof wall is laid down.

The objective therefore was to compare hoof growth rates at different locations along the hoof wall from the anterior to the posterior region of the bovine claw. In a controlled experiment, cattle were switched from a barley-based diet to a maize based diet (the isotopic spacing between the diets was 13.6‰). The cattle were maintained on this experimental diet for 168 days. To compare growth rates along the wall, three slices were taken *post-mortem* from one bovine claw. In addition, one claw from each of three different animals was sampled at different depths from the surface to determine any possible time lag (“offset”) in the laying down of keratin tissue layers. From each hoof as many as 41 superficial samples were taken over the first 60 mm, starting at the periople, and up to 12 samples were taken sequentially at increasing depths to a depth of 6 mm at five particular points on the surface. The growth rate of the abaxial wall of the bovine claw increased from the anterior to the posterior region of the bovine hoof. Analysis of the deep samples revealed that the deeper layers were younger than the surface layers. This offset was inversely related to the hoof growth rate, i.e. hooves with a high hoof growth rate showed a smaller offset. Observed offsets ranged between 9.2 ± 1.8 days per mm in depth for a high and 14.0 ± 2.8 days per mm in depth for a low hoof growth rate and were significantly different ($t \geq 3.92$, $p < 0.0005$, $n = 19$ or 27). The results of this study demonstrate that when sampling hooves or hoof fragments for applications such as diet reconstruction, careful consideration needs to be given to sample location.

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Turnover of carbon in bovine “soft” tissues

Stable isotope (SI) analysis of animal tissue has potential to authenticate the dietary history of meat animals. The use of SI ratio analysis (SIRA) to investigate the turnover of carbon and nitrogen in bovine *Longissimus dorsi* and *Psoas major* muscles was demonstrated in Animal Research Report 2006, p 71. Turnover data for the carbon of soft tissues are reported here. The diets of five groups ($n=10$ each) of continental crossbred beef cattle were switched from a control diet containing barley and unlabelled urea to an isotopically distinct diet containing maize and ¹⁵N labelled urea for 168, 112, 56, 28 or 14 days pre slaughter. A group of 10 animals fed the control diet for 168 days served as an experimental control. Samples of liver, heart, kidney and brain were collected immediately *post-mortem* and processed for SIRA.

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Based on the ^{13}C turnover curve of liver (Figure 45), kidney (Figure 46), heart (Figure 47) and brain (Figure 48), it is evident that after 168 days of feeding of the isotopic diet, the ^{13}C equilibrium was reached in liver and kidney and nearly reached in heart and brain. Based on the calculated mean composite (including the intake of straw) $\delta^{13}\text{C}$ values of $-28.5 \pm 0.02\text{‰}$ for the control diet and $-16.1 \pm 0.15\text{‰}$ for the isotopic diet after 168 days, the diet-tissue spacing (fractionation values) for liver, kidney, heart and brains tissues were higher in animals fed the control diet compared to the isotopic diet. In general, animal tissues have a $\delta^{13}\text{C}$ value $\sim 1\text{‰}$ more positive compared to that of diet.

The turnover rates of ^{13}C in bovine liver, kidney, heart and brain were determined by fitting the $\delta^{13}\text{C}$ data with exponential regression models and were 15.5, 18.3, 43.2 and 46.8 days, respectively. The ^{13}C turnover rates were faster (shorter half-lives) in liver and kidney compared to those of heart and brain. A fast isotopic turnover in bovine liver may be expected because liver is a highly metabolically active organ characterised by a rapid breakdown and synthesis of cellular compounds. Like liver, a similar level of metabolic activity is also expected in kidney and this is evident from the comparable ^{13}C turnover rates of bovine liver and kidney. In heart and brain tissues, the ^{13}C turnover rates were comparatively slower (than liver and kidney) but similar to each other. As in the heart, the ^{13}C turnover rate determined for bovine brain reflected a moderate level of metabolic activity in the brain tissue of beef cattle.

It is concluded that bovine liver and kidney had fast ^{13}C turnover rates while those of heart and brain were slower. Results demonstrated that in a period of 2-3 months of feeding, the $\delta^{13}\text{C}$ values of bovine liver and kidney would be $>90\%$ equilibrated with the diet. On the other hand, heart or brain need a period of approximately 6 months to reflect $>90\%$ of the $\delta^{13}\text{C}$ and value of the diet. Since the turnover rates were somewhat similar between liver and kidney and between heart and brain tissues, it is concluded that the combined isotope analysis of either liver or kidney and heart or brain could provide a similar level of temporal information about the timing of pre-slaughter diet shift in cattle.

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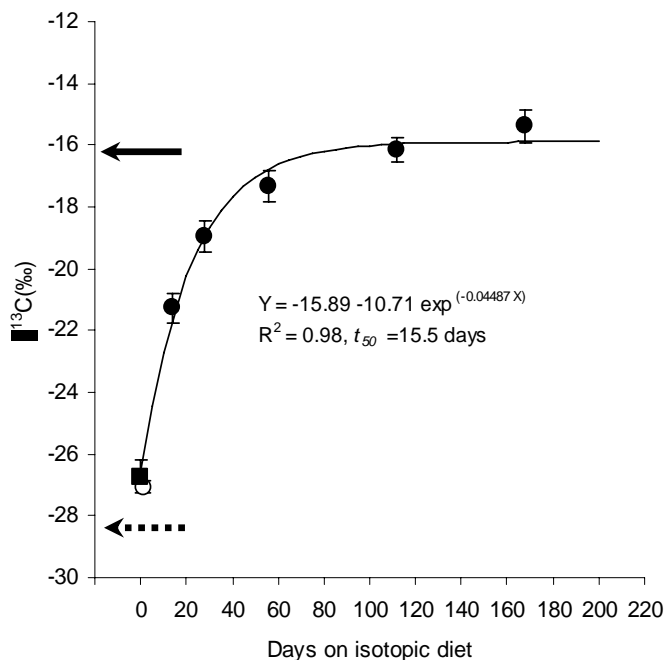


Figure 45. Turnover of ¹³C in bovine liver. Solid circles (●): cattle were on the isotopic diet for different periods (14, 28, 56, 112 and 168 days); solid square (■): cattle were on the control diet for 168 days; hollow circle (○): pre-experimental group. Error bar represents 1 standard deviation of mean (n=10). Composite isotopic values of the diet for the 168-day groups (T5 and T6) are indicated for the isotopic diet (solid arrow) and the control diet (broken arrow).

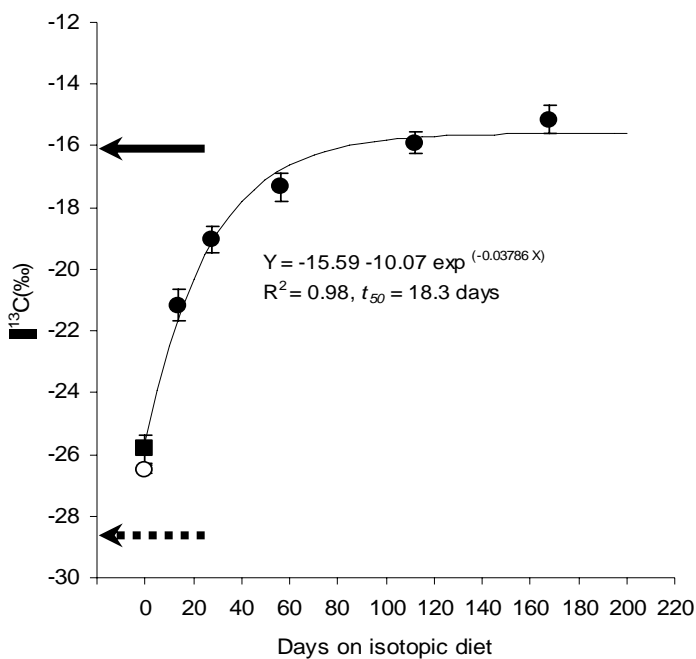


Figure 46. Turnover of ¹³C in bovine kidney. Solid circles (●): cattle were on the isotopic diet for different periods (14, 28, 56, 112 and 168 days); solid square (■): cattle were on the control diet for 168 days; hollow circle (○): pre-experimental group. Error bar represents 1 standard deviation of mean (n=10). Composite isotopic values of the diet for the 168-day groups (T5 and T6) are indicated for the isotopic diet (solid arrow) and the control diet (broken arrow).

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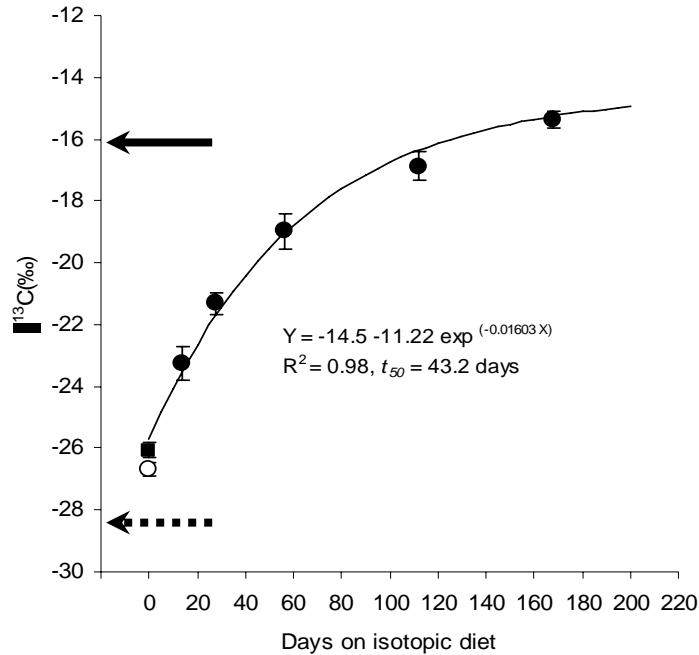


Figure 47. Turnover of ^{13}C in bovine heart. Solid circles (●): cattle were on the isotopic diet for different periods (14, 28, 56, 112 and 168 days); solid square (■): cattle were on the control diet for 168 days; hollow circle (○): pre-experimental group. Error bar represents 1 standard deviation of mean ($n=10$). Composite isotopic values of the diet for the 168-day groups (T5 and T6) are indicated for the isotopic diet (solid arrow) and the control diet (broken arrow).

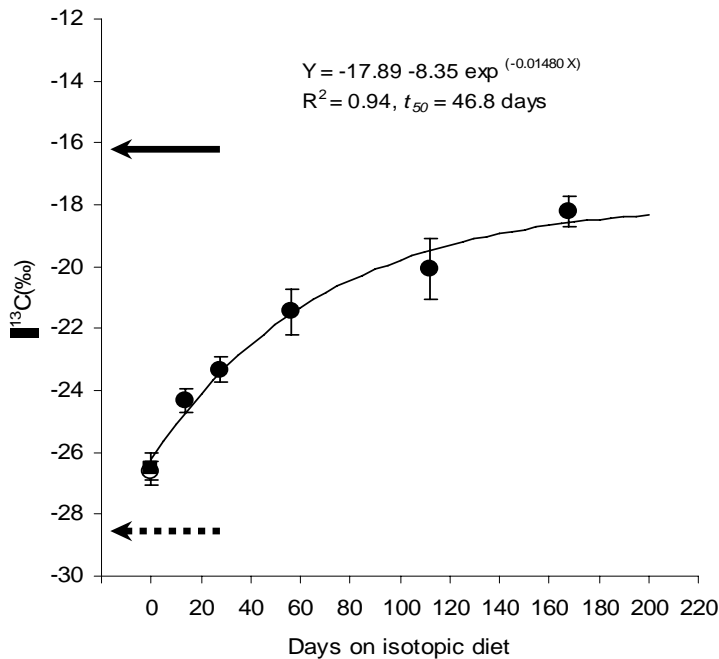


Figure 48. Turnover of ^{13}C in bovine brain. Solid circles (●): cattle were on the isotopic diet for different periods (14, 28, 56, 112 and 168 days); solid square (■): cattle were on the control diet for 168 days; hollow circle (○): pre-experimental group. Error bar represents 1 standard deviation of mean ($n=10$). Composite isotopic values of the diet for the 168-day groups (T5 and T6) are indicated for the isotopic diet (solid arrow) and the control diet (broken arrow).

The record of dietary change on the stable isotope composition of different sheep tissues

Animal movement often implies a change in the environment *via* the local climate or diet. This change can be recorded in the stable isotope composition of an animal's tissues under two conditions: (1) the two environments should be isotopically distinct; (2) tissue turn-over must be fast enough to record this change in its isotopic composition. Using sheep as a model, a study was undertaken with three objectives: (a) to measure the rate and magnitude of isotopic change following a change in diet in different animal tissues, (b) to examine the role of the main factors governing the rate and magnitude of this change including duration of exposure to the new diet and (c) the balance between tissue turn-over and tissue growth.

Thirty-six lambs born in the Livestock Research Centre, Teagasc, Athenry, in February-March 2006 were transferred to Grange Beef Research Centre where they were offered an isotopic distinct diet. The experimental diet contained a mix of concentrate (80 wt %) and maize silage (20 wt %). The concentrate was composed almost exclusively (~90 wt %) of C₄ plant material and was designed to meet requirements for energy and protein for a particular rate of growth. Feed allowances were calculated and readjusted to ensure a constant target weight gain of 150 g d⁻¹ and 50 g d⁻¹ for high ration (H) and low ration (L) lambs, respectively, and maintenance weight for the young and old ewes. Food was offered to each animal individually in one batch in the morning, together with unlimited access to tap water. All animals were weighed every two weeks. Animals were slaughtered at the beginning of the diet change (Time 0) and after 2, 4, 8, 14 and 22 and 33 weeks. Additional animals remained on the control ration until week 22 when half were slaughtered and half were offered the isotopically distinct ration for 4 weeks prior to slaughter.

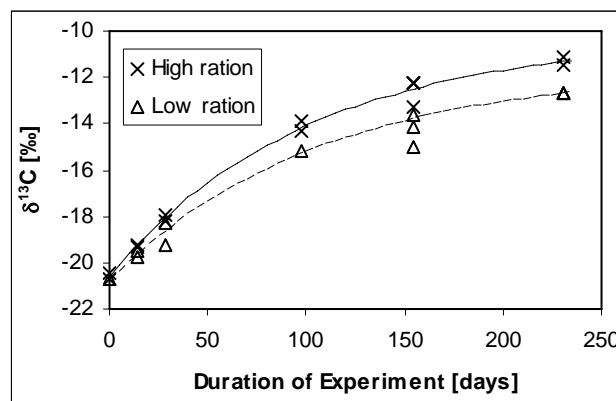


Figure 49. Protein turnover in *Longissimus dorsi* as recorded by C stable isotopes.

Stable isotope analysis (SIA) (C and N) of *Longissimus dorsi* (LD) muscle and diet samples has been completed. Statistical analysis of the data revealed no significant difference between sampling locations within one muscle. Figure 49 shows protein turnover in LD as recorded by C stable isotopes. The half-life of protein turnover in animals fed a high ration was estimated to be 69.6 days compared to an estimated half-life of 73.8 days for animals fed a low ration.

The following muscles have been prepared to determine inter-muscular variation of stable isotope values within one animal: *Longissimus dorsi*, *Psoas major*, *Biceps femoris*, *Cleidooccipitalis*

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(neck) and *Flexor digitorum superficialis* (calf muscle). All samples have been defatted, weighed and are awaiting SIA.

To investigate inter-tissue (muscle vs. lipid) variation in C turnover within a meat sample, as an approach to understanding previous feed history of animals, lipid extracted from the LD will be collected and separated into triglyceride and phospholipid fractions using solid phase extraction. Carbon turnover in both fractions will be determined using SIA. Subcutaneous adipose tissue attached to the LD will be also prepared for SIA and results compared to those of de-fatted LD and combined LD lipid fractions. SIA of muscle and lipid samples will be carried out at the University of East Anglia, England.

RMIS No. 5214 & 5430

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Effect of age and food intake on dietary carbon turnover in sheep wool

The reconstruction of animal feeding strategies is an important piece of the puzzle that links an animal to its environment. Stable isotope analysis has emerged as one of the most versatile tools available to animal ecologists to reconstruct animal dietary preferences. Recently, hair has received an increased interest as an archive of past diets. This is because hair grows continuously and becomes biologically inactive once formed. Therefore, measuring sections of hair facilitates the examination of feeding history at a fine scale. Although progress has been made in understanding the kinetics of isotope turnover in hair, the influence of age and animal growth rate on hair isotope turnover have not been investigated so far. It is important, however, to quantify this possible source of variability because there is usually no control on these parameters when sampling hair from wild animals. We hypothesised that young, growing mammals will record the isotope composition of their diet in keratin faster than older animals or animals fed at maintenance.

Three Suffolk cross ewes born between March and April 2005 and raised in Co. Carlow, Ireland, were selected for the experiment. The ewes were raised outdoors on pasture from weaning. Ewes were brought to Grange Beef Research Centre on the day prior to a switch to an experimental diet (28th August, 2006) and penned individually. Three purebred Suffolk ewes born in Athenry (Co. Galway) in March 2000 were also selected for the experiment. The ewes were on pasture for six months prior to the diet-switch and brought to Grange Beef Research Centre one day prior to the switch to an experimental diet (26th September, 2006) and penned individually.

From the lambs in the study, described on page 133 the following were selected for this study, one offered a high (H) allowance and one offered a low (L) allowance and slaughtered 153 d after the diet-switch; (2 H and 2 L) 231 d after the diet switch. The three young ewes and three old ewes were kept for 246 d and 218 d, respectively, on the experimental diet and then slaughtered.

Wool sampling – A 15 cm x 15 cm patch of wool was shorn off the side of the animals prior to diet-switch, using an electric clipper with a size 10 blade, leaving wool to a depth of 1.2 mm on the skin. This wool was discarded and a 1mm deep sample of wool was collected using a size 40 blade, leaving 0.2 mm on the skin. This 1 mm sample and subsequent samples were taken from a

5 cm x 5 cm square at the centre of the larger patch initially shorn, before cleaning the rest of the patch at the same 0.2 mm height. This two step shearing protocol was designed to prevent recently grown wool from mixing with “older” wool. Animals were shorn every 2-3 days during the first month following the diet-switch, then every week over the subsequent two months, then every two weeks to slaughter. Each sample thus represents a time-averaged wool growth between two shearing dates. Samples were processed according to previously published protocols and natural abundance stable isotope ratios of carbon ($^{13}\text{C}/^{12}\text{C}$) in sheep wool measured. Data were subjected to the reaction progress approach developed by Cerling *et al.* (2007) to determine turnover rate constants and fractions for each of the pools.

All lambs showed very little (<0.5‰) change in their wool $\delta^{13}\text{C}$ values over the two months preceding the diet-switch, suggesting that lamb wool was at or near equilibrium with the pre-experimental diet at the beginning of the experiment with an average $\delta^{13}\text{C}$ value of -20.5‰. The ewes exhibited lower values averaging -27.0‰ and -26.2‰ for the young and old ewes, respectively.

Figure 50 shows the $\delta^{13}\text{C}$ profiles measured for the H and L lambs (Figure 50A-B), the young ewes (Figure 50C) and the old ewes (Figure 50D). The x-axis is expressed in time units and $t=0$ is the time of diet-switch. Because each sample represents the regrowth since the last shearing event, the isotope data are plotted using the average date between two consecutive shearing episodes. The hair response to the diet-switch can be decomposed into three phases 1) a lag period ranging from 6 to 15 d with very little or no change in $\delta^{13}\text{C}$ values; 2) a fast increase in $\delta^{13}\text{C}$ values starting at the end of the lag until day ~30; 3) a slower increase from day 30 until the end of the experiment. The amplitude of isotopic change measured in wool ($\Delta^{13}\text{C}_{\text{wool}} = \delta^{13}\text{C}_f - \delta^{13}\text{C}_i$) was, on average, 10.8‰ for the L lambs, 11.6‰ for the H lambs, 16.6‰ for the young ewes and 15.2‰ for two out of the three old ewes. For comparison, the amplitude of isotopic change in diet ($\Delta^{13}\text{C}_{\text{diet}}$) was 11.6‰ for the lambs, 18.0‰ for the young ewes and 17.2‰ for the old ewes. Therefore, only wool from H lambs was in equilibrium with the animal's diet at the time of slaughter.

This study demonstrates for the first time that growth and age play a role in the record of dietary $\delta^{13}\text{C}$ value in sheep wool. The wool from fast-growing lambs approached equilibrium faster than the wool from slow-growing lambs and young ewes, with old ewes being the slowest. However, the differences between the four different groups of animals were relatively small suggesting that a single equation can be used to reconstruct previous diets for animals of different age, provided that the dietary make-up is not too different and that all individuals are fed at or above maintenance.

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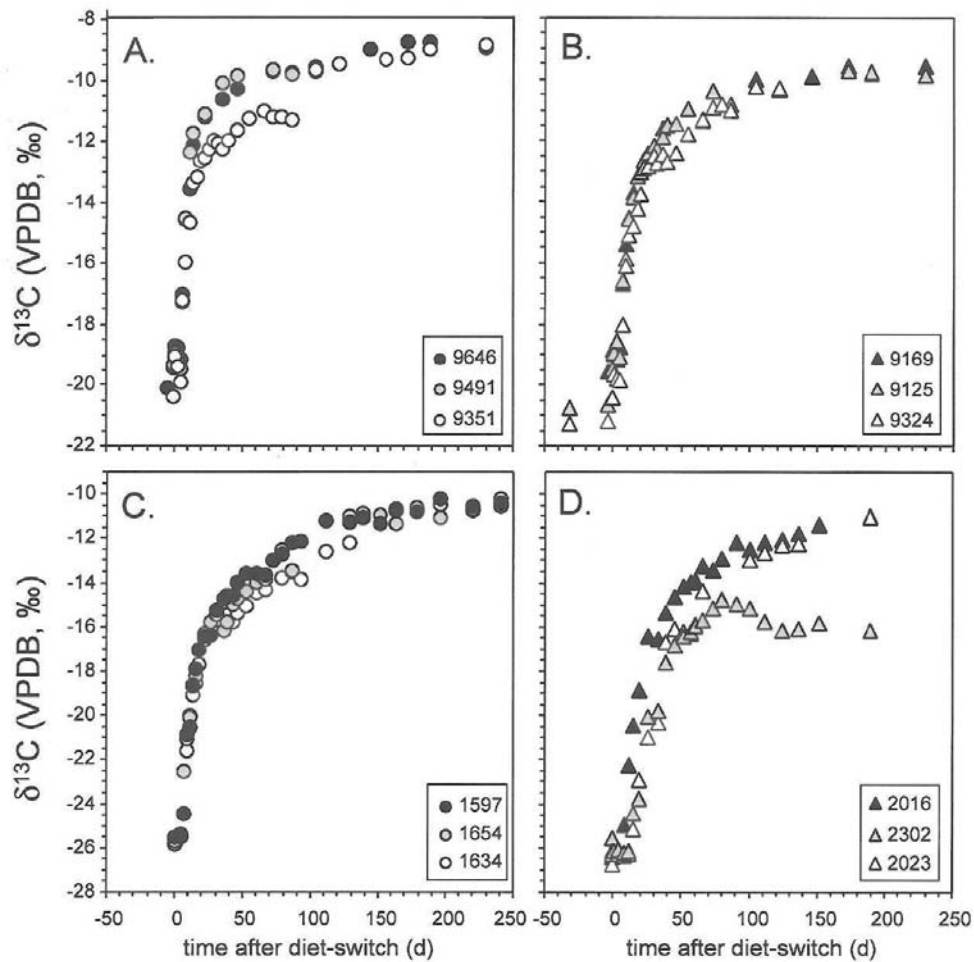


Figure 50. Wool $\delta^{13}\text{C}$ values versus time for the twelve study animals. A. High-ration lambs; B. Low-ration lambs; C. Maintenance young ewes; D. Maintenance old ewes.

RMIS No. 5430

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The change in the fatty acid composition of muscle from cattle that grazed perennial ryegrass when subsequently offered concentrate-based rations

Consumer interest in the relationship between diet and health has focussed attention on the composition of food and in particular on its fatty acid composition. Beef can be a source of polyunsaturated fatty acids (PUFA), particularly omega (n)-3 PUFA and conjugated linoleic acid (CLA) which are considered to be beneficial to human health (Simopoulos, 1999). While consumption of grazed grass by cattle results in higher concentrations of these fatty acids in muscle than consumption of conserved forage or concentrates (French *et al.*, 2000), feeding concentrate-based rations before slaughter may be required to achieve particular market specifications. Our objective was to determine the temporal pattern of the fatty acid composition when previously grazed cattle were offered a concentrate-based ration.

Sixty-three continental crossbred (Charolais × Limousin/Friesian) heifers (mean live weight of 387 [SD = 30.6] kg) were divided into three blocks on the basis of decreasing age. Within block, heifers were randomly assigned to 1 of 21 cells. Corresponding cells from each age block were grouped to give 21 groups of three heifers each. Groups were then randomly assigned to treatments (T) and slaughter dates (D). On April 26, six heifers which had been assigned to a pre-experimental group were slaughtered, forty-two heifers were turned out to pasture where they rotationally grazed six paddocks for 90 days (PAS) while fifteen heifers were housed (PH-CON) and offered 6kg concentrates and 2kg straw per head, daily. After grazing, PAS heifers were allocated to their pre-assigned T and D and housed in slatted floor pens, in groups of six according to treatment. Six of the PAS heifers and six of the PH-CON heifers were slaughtered to represent d0 of the indoor phase. The additional treatments were 6 kg of concentrate plus 2 kg of straw (PAS-CON), 4.2 kg of concentrate plus 6.4 kg of grass DM (PAS-GS50) and 1.5 kg concentrate plus 6.4 kg of grass DM (PAS-GRA). Heifers (n = 3/treatment, on each date) were slaughtered on d28, 56, 91 and 120 days thereafter. All heifers were weighed at intervals of approximately 3 weeks and dietary allowances were adjusted to maintain comparable growth rates and carcass fatness across treatments while avoiding feed refusals. *Longissimus* muscle, collected 24 h post-mortem, was stored at -30°C until fatty acids were extracted and measured as previously described. Data from the pre-experimental and d0 groups were subjected to analysis of variance with block and T as sources of variation. Linear modelling approaches were used to determine the pattern of change of tissue fatty acid composition over time.

Compared to the pre-experimental cattle, grazing for 96 days increased the proportion of CLA (P=0.07) and n-3 PUFA (P<0.05) in muscle lipids (from 7.6 to 10.3g/kg) (sed 1.51) and from 14.2 to 16.8g/kg (sed 2.01), respectively. At housing, the proportion of CLA and n-3 PUFA was lower (P<0.05) (6.6 and 10.9g/kg, respectively) in muscle lipids of PH-CON heifers.

The change in muscle CLA was described by:

PAS-GS50: $y = -0.101$ (se 0.0466) $X + 0.0007$ (se 0.00039) $X^2 + 10.5$ (se 1.00), MSE 5.44, R^2 19.4, P = 0.097.

PAS-CON: $y = -0.085$ (se 0.0313) $X + 0.0005$ (se 0.00026) $X^2 + 10.2$ (se 0.67), MSE 2.56, R^2 43.7, P<0.01.

PH-CON: $y = -0.017$ (se 0.0081) $X + 6.3$ (se 0.58), MSE = 2.23, R^2 21.2, P = 0.05.

Where y = CLA (g/kg fatty acids), x = days post housing (d 96-d 216) and MSE = mean square error.

The pattern of loss of CLA in PH-CON from the beginning of the study was described by: $y = -0.015$ (se 0.0057) $X + 7.7$ (se 0.744), MSE = 4.10, R^2 24.3, P <0.05.

The CLA proportion in muscle lipids of PAS-GRA heifers averaged 11.2, 11.4, 7.5 and 10.0g/kg fatty acids at 28, 56, 91 and 120 days post-housing but no pattern was evident.

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The change in muscle n-3 PUFA was described by:

PAS-GS50 : $y = -0.001$ (se 0.0006) $X + 0.061$ (0.0771) $X^2 + 16.0$ (se 1.66), MSE 14.88, R^2 20.8, $P = 0.09$.

PAS-CON: $y = -0.002$ (se 0.0010) $X + 0.129$ (se 0.1140) $X^2 + 17.2$ (se 2.49), MSE 33.78, R^2 26.2, $P < 0.05$.

PAS-GRA: $Y = -0.45$ (se 0.447) $X + 0.02$ (se 0.019) $X^2 - 0.0004$ (se 0.00025) $X^3 + 0.000002$ (se 0.0000011) X^4 , MSE 20.01, R^2 28.2, $P = 0.09$.

The n-3 PUFA proportion in muscle lipids of PH-CON heifers averaged 14.6, 6.9 and 10.3g/kg fatty acids after 124, 187 and 216 days at the study without a clear pattern of change.

Replacement of grazed grass with a concentrate-based ration resulted in a decrease in the CLA and n-3 PUFA proportion of muscle lipids that could be described by a quadratic function. Feeding cut grass, maintained the CLA and n-3PUFA proportion in muscle of previously grazing cattle. The modest proportion of the variation in CLA (and n-3PUFA) explained by the models presented reflects the variation in muscle fatty acid *per se*.

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Fatty acid composition of *Longissimus* muscle from grazing or concentrate-fed cattle supplemented with sunflower seeds and fish oil

Effects of supplementation of grazing cattle, or cattle offered a linseed oil-fortified concentrate, with a blend of sunflower seeds and fish oil (SSF) on the fatty acid profile of muscle, with emphasis on the conjugated linoleic acid (CLA) and vaccenic acid (VA) proportions were examined. Sunflower seeds and oil were also compared in grazing cattle. Charolais crossbred heifers of initial bodyweight 366 (s.d. 35.0) kg were offered (n = 10/treatment): grazed grass (G), G supplemented with 2.5 kg/d SSF (293g sunflower seeds and 67 g fish oil/kg) (GSSF), G and 2.5 kg/d of a supplement containing 155 g sunflower oil and 67 g fish oil/kg (GSOFF), concentrates containing 37g linseed oil/kg (C) or C and 2.5 kg/d SSF. Cattle were slaughtered after 150 days and fatty acid methyl esters prepared from the neutral (N) and polar (P) lipid fractions of the *Longissimus Dorsi* muscle were analysed. Data were subjected to ANOVA and "a priori" contrasts were used to evaluate the effects of ration type, lipid supplementation and source of sunflower oil. Carcass weight averaged 253, 261, 264, 270 and 258 (sed 3.3) kg, for G, GSSF, GSOFF, C and CSSF, respectively. The N fraction of muscle from G, GSSF, GSOFF, C and CSSF-fed cattle had 1.31, 1.62, 1.92, 1.08 and 1.03 (sed 0.040) g cis-9, trans-11 CLA /100g fatty acids, respectively. The corresponding values were 4.10, 5.52, 7.27, 2.94 and 5.84 (sed 0.260) for VA, 1.06, 1.24, 1.26, 1.51 and 1.60 (sed 0.040) for 18:2 n-6 and 0.64, 0.45, 0.44, 0.67 and 0.44 (sed 0.010) for 18:3 n-3. The P fraction of muscle from G, GSSF, GSOFF, C and CSSF had 0.43, 0.47, 0.57, 0.27 and 0.16 (sed 0.010) g cis-9, trans-11 CLA /100g fatty acids, respectively. The corresponding values were 1.00, 1.17, 1.68, 0.97 and 2.22 (sed 0.090) for VA, 9.9, 13.9, 13.5, 18.2 and 20.9 (sed 0.33) for 18:2 n-6, 3.90, 2.36, 2.68, 3.69 and 1.96 (sed 0.060) for 18:3 n-3, 2.40, 2.23, 2.13, 1.69 and 2.83 (sed 0.080) for 20:5 n-3 and 0.27, 0.66, 0.65, 0.25 and 0.62 (sed 0.030) for 22:6 n-3. It is concluded that (1) despite comparable 18:3 n-3 intakes across treatments, muscle lipids of pasture-fed cattle contained higher VA, total and cis-9, trans-11 CLA proportions (2) supplementation of pasture resulted in larger marginal increases in total and cis-9, trans-11 CLA but smaller increases in VA proportions than with supplementation of concentrate and, (3) sunflower oil induced a higher enrichment of VA, total and cis-9, trans-11 CLA in muscle relative to sunflower seeds.

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Vitamin E inclusion in oil-supplemented grass- or concentrate-based diets: effects on beef colour characteristics in a high oxygen atmosphere during retail display

The source of lipid, e.g. sunflower, linseed or fish oil, and the basal diet, i.e. grass or concentrate, fed to ruminants affects the fatty acid composition of their meat (De La Torre *et al.*, 2006). Increasing the concentrations of conjugated linoleic acid and the highly oxidisable polyunsaturated fatty acids in meat may predispose it to discolouration. Supranutritional supplementation with vitamin E (VE) may be required to counteract this. The objective was to determine the effect of vitamin E inclusion in grass- or concentrate-based cattle diets supplemented with combinations of sunflower seeds/oil and fish oil on the colour stability of beef.

Ninety continental cross-bred heifers (mean initial live weight = 366 kg, SD = 35 kg) were assigned to two finishing systems, an outdoor grazing system and an indoor concentrate-based system. The indoor concentrate finishing system consisted of a control group (C) which received 6 kg/425 kg bodyweight of a base ration formulated to simulate linolenic acid supply from grass; the other 4 indoor treatments received 70% of the base ration and 2.5kg supplementary concentrates with 1.09kg sunflower seed + 0.17kg fish oil but with 0 (CS0), 200 (CS500), 400 (CS1000) or 1200 (CS3000) I.U. of VE per kg. The outdoor finishing system consisted of: unsupplemented grass (G); two further treatments with restricted grazing and a dietary supplement of 2.5kg concentrates that supplied 1.09kg sunflower seed + 0.17kg fish oil but with 0 or 400 I.U. VE (GS0 and GS1000, respectively) and 0.34kg sunflower oil + 0.17kg fish oil + 400 I.U. VE per kg (GO1000). Following slaughter, samples of *M. longissimus dorsi* (LD) were excised from the 12th rib on one side of the carcass and divided into steaks (2.5cm thick); samples of *M. psoas major* (PM) were also recovered. All samples were frozen at -20°C for eight months prior to retail display. Following thawing, 5 steaks were cut from each muscle, packaged in a high O₂ atmosphere and placed in a refrigerated (<5°C), lighted (2800lm) retail display cabinet for 10 days. Hunter redness ('a') and yellowness ('b') were measured on days 0, 2, 4, 8 and 10. Saturation ('C') was calculated as $[a^2 + b^2]^{0.5}$. Colour data were analysed as a split-split-split plot design with main effects of diet, muscle, time and all interactions included in the model. The VE data were analysed as a split-split plot design.

There were treatment × time and muscle × time interactions for 'a' (both P<0.001) and 'C' (P= 0.009 and P<0.001, respectively) values. For both redness and saturation, values decreased between days 0 and 2, days 2 and 4 and days 4 and 8 (all P<0.05). There were no significant differences between treatments on day 0 or 2 except that CS500 was more saturated than G on day 0 and more red than G on day 2 (both P<0.05). For redness, CS500 was more red and more saturated (both P<0.05) than CS0, CS1000, GS0, GS1000 and GO1000 on day 4 but not on days 8 or 10. CS3000 was more red (P<0.05) than CS0, CS500 and CS1000 on day 8, but not different from G or GS1000. Thus, while increasing VE is an effective means of improving shelf-life of beef from concentrate-fed cattle, offering G is as effective as 3000 I.U. •hd⁻¹•d⁻¹ of VE. There was a treatment × muscle interaction for muscle VE concentrations (Figure 51).

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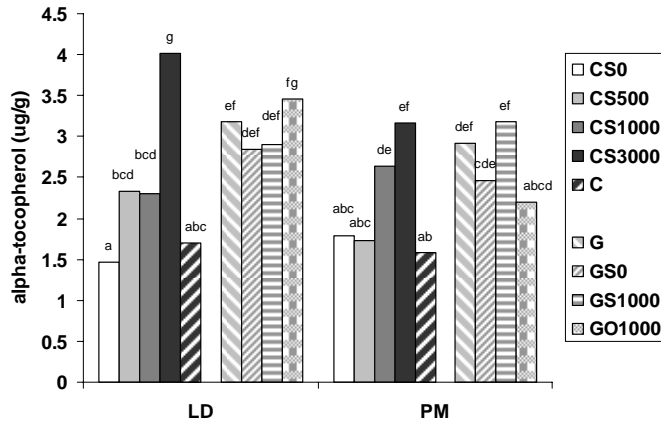


Figure 51. Alpha-tocopherol (VE) concentrations ($\mu\text{g/g}$) in LD and PM muscles (treatment \times muscle, $P=0.016$; $\text{SED}=0.3944$).

For LD, all G treatments had higher ($P<0.05$) α -tocopherol concentrations than C treatments, with the exception of CS3000, which tended to be the highest and was not significantly different from GO1000. For PM, G had a higher ($P<0.05$) α -tocopherol concentration than C, CS0 and CS500 but was not different from CS1000 or CS3000. For PM also, GO1000 was lower ($P<0.05$) than GS1000 but for LD, GO1000 tended to be higher than other G treatments.

It is concluded that for concentrate feeding systems, the beneficial effects on redness and saturation of increasing the dietary supply of VE become more apparent as the display period progresses but that grazing is as effective as a targeted VE intake of $3,000 \text{ I.U.} \cdot \text{head}^{-1} \cdot \text{d}^{-1}$ on a concentrate-based diet in terms of colour stability.

Delta 9 desaturase gene expression in muscle, adipose tissue and liver of beef heifers following supplementation of grass with a concentrate containing sunflower seed and fish oil.

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Delta 9 desaturase gene expression in muscle, adipose tissue and liver of beef heifers following supplementation of grass with a concentrate containing sunflower seed and fish oil

New dietary strategies to increase the conjugated linoleic acid (CLA) content of ruminant derived foods are receiving considerable attention because of the potential benefits for human health of increased CLA consumption. In ruminant tissues delta 9 desaturase is involved in the endogenous synthesis of CLA from vaccenic acid (VA), formed during rumen microbial biohydrogenation of linoleic and linolenic acid. Previous experiments have shown that dietary sunflower and fish oil inclusion have increased the concentration of CLA in bovine tissue. The objective of this experiment was to examine the effects of grazed grass or concentrates either alone or supplemented with sunflower seed and fish oil on the expression of the delta 9 desaturase gene in liver, subcutaneous adipose tissue and muscle of fattening beef heifers.

Forty Charolais or Limousin crossbred heifers of similar nutritional history were blocked on breed and liveweight and randomly assigned to two outdoor and two indoor groups ($n=10/\text{group}$).

The outdoor animals were either offered unsupplemented pasture (*Lolium Perenne*), or restricted pasture supplemented with 2.5kg of sunflower seed (29%) and fish oil (6%), while indoor groups were fed a basal concentrate rich in linolenic acid (32% of total fatty acids) or restricted basal concentrate with 2.5kg of the sunflower seed and fish oil based supplement. Animals were slaughtered after approximately 150 days on experimental ration treatments. Samples of subcutaneous adipose, liver and muscle were collected within 45 minutes of slaughter and stored in RNALater at -70°C before total RNA extraction using RNAeasy Mini kits (Qiagen). Total RNA extracts were quantified using a spectrophotometer and c-DNA was synthesised using Invitrogen Superscript III First-Strand Synthesis System for RT-PCR. Quantities of mRNA were determined relative to 18S-RNA using quantitative real time-RT PCR. Data were analysed using the GLM procedure of SAS. Delta 9 desaturase mRNA levels were significantly lower ($P<0.05$) in muscle and subcutaneous adipose of grass-fed outdoor animals compared to concentrate-fed animals but were unchanged in liver ($P>0.05$). Supplementation of the diet with sunflower seed and fish oil had no effect on delta 9 desaturase gene expression in any tissue examined. These results show that grass-based diets result in lower delta 9 desaturase gene expression in muscle and adipose tissue of beef animals despite increasing overall CLA levels in the tissues.

RMIS No. 5409

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The impact of a diet with CLA – enriched beef on the diabetic phenotype in ob/ob mice

The Metabolic Syndrome defines a clustering of metabolic irregularities, including obesity, insulin resistance and dyslipidemia, which is associated with a high risk of type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD). Conjugated Linoleic Acid (CLA) refers to a family of positional and geometric isomers of linoleic acid (LA; C18:2 n-6). Animal feeding studies have shown that synthetic forms of the cis-9, trans-11 isomer of CLA (c9,t11-CLA) reduces the risk of T2DM and CVD, decrease cholesterol and triacylglycerol (TAG) concentrations, inhibit the development of atherosclerosis, and improve insulin sensitivity. To date all of the work in this area has focused on synthetic CLA sources. The natural dietary sources of CLA are ruminant milk and meat, where most of the CLA present is in the form of the c9,t11-CLA isomer. Therefore, the aim of this study was to investigate the effect of high c9,t11-CLA beef (produced by supplementing grazing cattle with sunflower oil and fish oil) on risk factors associated with the metabolic syndrome and to determine its efficacy relative to the synthetic form of the fatty acid. Twenty-two male ob/ob mice were randomly assigned to one of the three dietary treatments as stated in Table 69 for a 28 day period.

Whole body metabolic markers of insulin resistance and T2DM are presented in Table 69. Both the CLA-enriched beef and synthetic CLA interventions significantly reduced plasma glucose and NEFA levels compared to the linoleic acid and *trans*-vaccenic acid (TVA) diets ($p<0.05$). Whilst insulin concentrations were not significantly altered by the CLA diets, the HOMA index, which is a measure of insulin resistance was significantly improved by both CLA interventions ($P< 0.05$). There was no difference in cholesterol and triglyceride levels between any of the treatment groups.

Analysis of key inflammatory markers in the plasma demonstrated that IL-6 levels were significantly decreased in animals on the CLA diets ($p<0.05$). Adiponectin, which is involved in the regulation of lipid and glucose metabolism, and is known to be decreased in patients with T2DM was significantly increased in both groups of animals receiving the CLA diets ($p<0.05$) (Table 70).

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Table 69: The effect of CLA enriched beef on metabolic markers of T2DM in ob / ob mice

	<u>Control</u>	<u>Synthetic CLA</u>	<u>Beef CLA</u>	<u>TVA</u>
Glucose (mmol/L)	19.63 (0.076)	14.07* (1.04)	15.28* (1.49)	18.08 (0.77)
Insulin (pg/mL)	3005 (1359)	3258 (1398)	4027 (1606)	2823 (1439)
HOMA - IR	2.46 (0.19)	1.50* (0.29)	1.21* (0.14)	2.09 (0.17)
NEFA (mmol/L)	0.70 (0.029)	0.36* (0.032)	0.453* (0.025)	0.662 (0.24)
Cholesterol (mmol/L)	5.14 (0.42)	5.83 (0.17)	5.86 (0.46)	5.66 (0.504)
TAG (mmol/L)	1.49 (0.39)	1.39 (0.2)	1.37 (0.15)	1.51 (0.31)

Values reported represent group means with standard deviations in parenthesis. P<0.05

Table 70: The effect of CLA enriched beef on inflammatory markers in ob/ob mice

	<u>Control</u>	<u>Synthetic CLA</u>	<u>Beef CLA</u>	<u>TVA</u>
IL-6 (pg/ml)	1626.38 (138)	1151.24* (109)	1167.67* (168)	-
Adiponectin (mg/ml)	7198.2 (635)	9211.94* (706)	9961.05* (226)	-
Resistin (pg/ml)	5155.6 (3911.1)	1912.8 (176.7)	2214.3 (231.9)	1845.7 (192.9)
TNF α (pg/ml)	5.0 (0.50)	5.0 (0.54)	5.5 (0.47)	5.86 (0.74)
PAI-1 (pg/ml)	1892.0 (844.3)	4322.5 (852.7)	4421.1 (1547.2)	3539.4 (788.5)
MCP-1 (pg/ml)	153.9 (17.6)	193 (30.6)	185.9 (17.3)	184.1 (28.8)

The results of this study suggest that beef enriched with CLA may have beneficial effects on glucose metabolism, insulin sensitivity and mediators of inflammation, which are all key metabolic markers of T2DM and the metabolic syndrome.

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Indicators of innate and adaptative immunity in animals raised either on pasture or fed a concentrate-based diet indoors

Cattle at pasture are considered by consumers to have better welfare than those conventionally raised in confinement. However, few objective measurements have been made in this context. The objective of the study was to investigate indicators of innate and adaptative immunity in animals raised either on pasture or fed a concentrate-based diet indoors.

Charolais x Limousin heifers were randomly assigned at 8 months of age to pasture (grass-raised, n=10) or to an indoors concentrate-based diet (concentrate-raised, n=10). Animals were assigned to treatments for 10 months and blood samples were collected at 14 months of age (summer) and 18 months of age (winter). Lymphocytes were isolated on a Ficoll density gradient and resuspended in RPMI-1640 containing non-essential amino acids, 5% fetal calf serum and gentamycin 50 µg/ml. Lymphocytes (2×10^5 cells) were cultured in triplicate with 5 µg/ml concanavalin A (ConA), 20 µg/ml lipopolysaccharide (LPS, *E. coli* O111:B4) or PBS used as a negative control. After 48 h incubation at 37°C under 5% CO₂, 3.75 µCi [³H]-thymidine was added and plates were incubated for another 24 h. The stimulation index (SI) is defined as the mean CPM (count per minute) of the response of the antigen-stimulated cells divided by the mean of the response of PBS control cells. The phagotest kit (*Orpegen*) measuring the uptake of opsonized, FITC-labeled *E. coli* was used to quantify *in vitro* phagocytosis activity. Data were collected from 20,000 cells per sample by flow cytometry (Dako CyAn ADP). Results are reported as the percentage of phagocytosing cells in the neutrophil and monocyte gates and as the increase in the geometric mean of the green fluorescence (correlate with the average number of ingested bacteria per cell) of the gated cells, respectively.

Data were analysed to assess statistical differences between treatment groups using Student's unpaired t-test.

There was no difference ($P > 0.05$) in the percentage of leucocyte subpopulations (neutrophils, eosinophils, monocytes and lymphocytes) between the two treatments. An overall trend towards a decrease in the percentage of neutrophils and monocytes engaged in phagocytosis was observed in grass-raised beef (reach significance in winter for neutrophils, $P < 0.05$, Figure 52A and B). A further reduction in the phagocytosis activity has also been shown (lower number of ingested bacteria in neutrophils, 33.4% and 19.54% decrease in summer and winter respectively, $P < 0.005$, Figure 52C and D).

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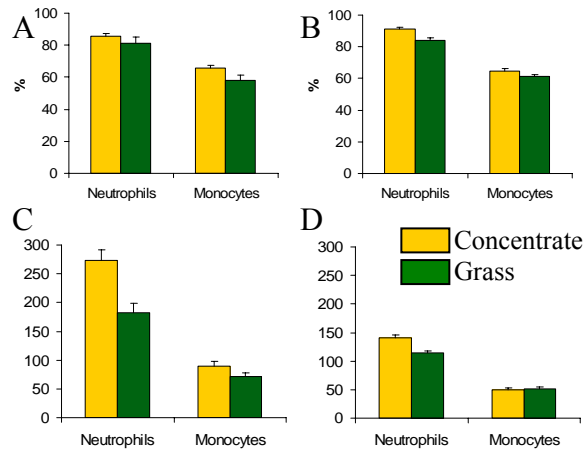


Figure 52. Phagocytosis capacity of neutrophils and monocytes. Percentage of cells engaged in phagocytosis during summer (A) and winter (B). Average number of bacteria ingested per leukocyte during summer (C) and winter (D).

In contrast, proliferation following ConA and LPS stimulation of lymphocytes isolated from grass-raised animals tend to be greater compared with samples from concentrate-raised animals ($P < 0.1$ in winter, Figure 53).

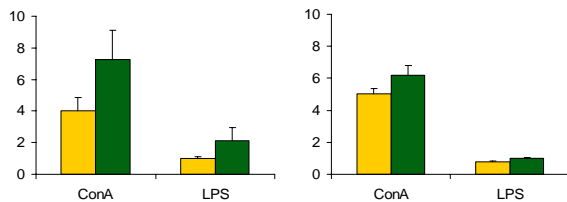


Figure 53. Lymphocytes proliferation upon ConA and LPS stimulations during summer (A) and winter (B).

The results of this study indicate an interaction of environment and diet on the immune system. The non-specific immune response, *e.g.* phagocytic activity of neutrophils, was greater in the intensive concentrate fed animals. Proliferative response involved in adaptive immunity could be enhanced in animals fed a grass diet. Further work has to be done to determine whether these differences could change animal response to disease.

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