

## NUTRITION AND PRODUCT QUALITY

### **Rumen fermentation and plasma metabolites in steers offered concentrates differing in energy source either as a supplement to grass silage or *ad libitum***

The wide variety of feed ingredients used in beef rations in Ireland means that isoenergetic and isonitrogenous concentrates can contrast from having high starch (of varying rumen degradability) to high digestible fibre concentrations and consequently a variable balance of nutrients for absorption. Due to changing relative costs of feeds and changing beef production systems, higher levels of concentrate feeding to beef cattle may be desirable. A particular concern with feeding high-grain diets however, is the excessively rapid fermentation of the high levels of starch to organic acids possibly resulting in acidosis.

The objective of this study was to examine the effects of concentrate ingredient composition and feeding level on rumen fermentation parameters and blood metabolites in beef cattle.

Rumen fermentation was determined using 4 rumen-fistulated steers (661 kg) in two consecutive (i. supplemented (SUP) and ii. *ad libitum* (AL) concentrate (C) feeding) 4 (diets) × 4 (14 d periods) Latin square design experiments. For SUP, grass silage (GS) was offered *ad libitum* plus 6.0 kg of C per head once daily. For AL, C was offered *ad libitum* plus 1.2 kg DM of GS daily. The 4 C were: rapidly fermentable starch (barley)-based (RFS), slowly FS (maize)-based (SFS), RFS + fibre-based (RFS+F) and fibre (pulp)-based (F). On d 11, rumen fluid samples were obtained at 0, 1 (SC only), 2, 4, 6, 8, 12, 16 (SC only) and 24 h post-feeding. On d 14, blood samples were obtained at 0, 3 and 6 h post-feeding. When offered SUP, there was no effect ( $P>0.05$ ) of C type on rumen pH, ammonia, lactic acid or total volatile fatty acid (VFA) concentrations or molar proportions of acetate, propionate and butyrate. When offered AL, rumen pH, lactic acid or total VFA concentrations and molar proportion of butyrate did not differ between C but the molar proportion of acetate was lower ( $P<0.05$ ) for RFS and SFS than RFS+F and F and the molar proportion of propionate was higher ( $P<0.05$ ) for RFS than RFS+F and F, with SFS being intermediate. Rumen ammonia concentrations were highest ( $P<0.05$ ) for SFS and lowest for RFS+F and F with RFS being intermediate. Plasma beta-hydroxybutyrate, urea and glucose did not differ ( $P>0.05$ ) between the C.

In conclusion, concentrate energy source had no effect on rumen pH or fermentation parameters when offered as a supplement to grass silage but significantly altered the end products of rumen fermentation when offered *ad libitum*.

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### **Rumen fermentation, microbial protein synthesis and nutrient flow to the omasum in beef cattle offered grass silage, maize silage and whole-crop wheat**

Beef cattle rarely consume sufficient grass silage to achieve their production potential and as a result supplementation with energy-rich concentrates is routine. Alternative ensiled forages are limited in many parts of Northern Europe but recent advances in plant breeding, agronomic practices and forage conservation technologies has meant an increase in the use of maize and whole-crop cereals for feeding beef cattle. Previous studies have generally reported that feeding maize silage either *ad libitum* or as a mixture in a grass silage-based diet increased dry matter intake (DMI) and performance of beef cattle and dairy cows. In contrast, several studies have shown an increase in DMI without an increase in performance when similarly feeding whole-crop wheat (WCW) silages preserved via fermentation or urea-treatment to beef cattle and dairy cows. Furthermore, a number of studies have found no difference in the performance of beef cattle offered fermented WCW and urea-treated WCW harvested at the same stubble height despite a higher DMI of the latter. Whole-crop cereal

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and maize silage constitute a less homogeneous material compared to grass silage as they contain grains rich in starch and straw/stovers with high fibre contents, the proportions of which can vary substantially. To better explain the responses of beef cattle offered maize and WCW silages, a more comprehensive understanding of the effects of these forages on digestion is required but currently only limited data are available.

The objective of this experiment was to determine within a single experiment the intake, rumen fermentation pattern, microbial protein synthesis and nutrient flows to the omasum in beef cattle offered grass silage, maize silage, fermented WCW and urea-treated unprocessed WCW.

Four ruminally fistulated Holstein-Friesian steers were used to examine the effects of feeding grass silage (GS), maize silage (MS), fermented whole-crop wheat (FWCW) and alkalage (urea-treated processed whole-crop wheat) (UPWCW), each supplemented with 3 kg of concentrate, on feed intake, rumen fermentation, microbial protein synthesis and nutrient flow to the omasum. The experiment consisted of a 4 × 4 Latin Square with each period lasting 21 days. The omasal sampling technique in combination with a triple marker method was used to measure flows to the omasum with Co-EDTA, Yb-acetate and indigestible neutral detergent fibre (NDF) as liquid, small and large particle phase markers, respectively. Microbial nitrogen (N) flow was assessed using purine bases as markers. Forage and total DMI was lower ( $P < 0.01$ ) for GS than MS, FWCW and UPWCW which did not differ ( $P > 0.05$ ). Rumen pH, lactic acid concentration and proportion of valeric acid were not affected ( $P > 0.05$ ) by forage. The rumen concentration of ammonia N ( $P < 0.001$ ) and molar proportion of acetic acid ( $P < 0.05$ ) were lower and the molar proportion of butyric acid higher ( $P < 0.05$ ) for MS. The UPWCW produced the highest concentration of ammonia N ( $P < 0.001$ ) and acetate to propionate ratio ( $P < 0.05$ ) in the rumen. The apparent ruminal digestion of organic matter (OM) was lower ( $P < 0.05$ ) for MS, FWCW and UPWCW than GS. Intake of neutral detergent fibre (NDF) did not differ ( $P > 0.05$ ) between the diets but NDF ruminal digestibility was higher ( $P < 0.01$ ) for GS than the other forages which were similar ( $P > 0.05$ ). Total tract NDF digestibility was lower ( $P < 0.05$ ) for UPWCW than the other forages with GS highest and MS and FWCW being intermediate. Starch intake was lower ( $P < 0.001$ ) for GS than the other forages but there was no effect ( $P > 0.05$ ) of forage on omasal starch flow or ruminal digestibility. The flow of non-ammonia N and microbial N was higher ( $P < 0.05$ ) for MS, FWCW and UPWCW than GS. The efficiency of microbial N synthesis was higher ( $P < 0.05$ ) for FWCW than GS and MS with UPWCW intermediate. Plasma beta-hydroxybutyrate concentrations were highest with MS and lowest for GS ( $P < 0.001$ ) while concentrations of plasma urea were lowest for MS and highest for UPWCW ( $P < 0.001$ ).

In conclusion relative to grass silage, non-grass conserved forages can significantly increase feed DMI, alter the site of digestion and the capture of N in the rumen in beef cattle.

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### **Intake, rumen fermentation and nutrient flow to the omasum in beef cattle offered grass silage fortified with sucrose and/or supplemented with concentrate**

Grass silage is generally characterised by having low concentrations of water soluble carbohydrates (WSC) and a high proportion of soluble non-protein nitrogen (N). The former are low due to their fermentation during ensilage to products that will make a relatively small direct energy contribution to rumen fermentation. As the rate of degradation of the N products of ensilage in the rumen is high these characteristics can result in a potential energy imbalance within the rumen and lead to an inefficient capture of silage nitrogen by rumen micro-organisms, low yields of microbial protein and an increased loss of N. This excretion

of N to the environment is undesirable, particularly with the progressive constraints of environmental legislation. Synchronising the supply of energy and N release in the rumen has been suggested to improve the efficiency of microbial growth and animal performance. Research has shown that carbohydrate, and sugar in particular, supplementation of grass silage can improve the efficiency of microbial protein synthesis. For this reason there has been increased interest in selecting grass cultivars with elevated concentrations of WSC and of subjecting them to ensilage technologies that restrict WSC catabolism. Grasses of high WSC concentration had a better efficiency of N use in dairy cows, higher live-weight gain in lambs and beef cattle and higher DMI in beef cattle compared to lower WSC grasses. The principle underlying the benefits of elevated WSC in grass on ruminal N use efficiency should apply equally or more so to silage where the ratio of WSC to soluble NPN is generally more unfavourable. In farming practice, grass silage is generally not offered alone to beef cattle, and concentrate supplementation is used to overcome deficiencies in nutrient supply. The latter can also positively impact on the efficiency of N use and on animal performance. Therefore, the potential interaction between silage WSC and concentrate supplementation needs to be quantified.

The objective of this study was to determine the relative effects of an increase in grass silage sucrose concentration and/or supplementation of a starch-based concentrate on rumen fermentation and nutrient supply to the omasum in beef cattle.

Four ruminally cannulated Holstein-Friesian steers were offered grass silage only (G), G plus 3 kg concentrates/d (GC), G plus 90 g sucrose/kg dry matter/d (DM) (GS) and G plus 90 g sucrose /kg DM/d plus 3 kg concentrates/d (GCS) in a 4 × 4 Latin Square designed experiment. Omasal flow was estimated using Co-EDTA, Yb-acetate and indigestible neutral detergent fibre (NDF) as digesta flow markers and purine bases as microbial markers. Concentrate supplementation reduced ( $P < 0.01$ ) silage and increased ( $P < 0.001$ ) total DM intake whereas sucrose had no effect ( $P > 0.05$ ). There was a sucrose × concentrate interaction ( $P < 0.05$ ) for rumen pH, whereby the addition of sucrose to grass silage alone decreased pH, but to grass silage plus concentrate had no effect. Rumen ammonia nitrogen (N) ( $P < 0.01$ ), total VFA concentration ( $P < 0.05$ ) and the molar proportions of valerate ( $P < 0.05$ ) and butyrate ( $P < 0.001$ ) increased with concentrate supplementation, whereas sucrose supplementation had no effect ( $P > 0.05$ ) on rumen fermentation parameters. Organic matter (OM) intake, omasal OM flow, the quantities of OM apparently digested (OMAD) and truly digested (OMTD) in the rumen ( $P < 0.001$ ) and total tract OM digestibility ( $P < 0.01$ ) increased and apparent and true ruminal OM digestibility decreased ( $P < 0.05$ ) with concentrate supplementation. Supplementation with concentrate decreased ( $P < 0.05$ ) ruminal neutral detergent fibre (NDF) digestibility and increased ( $P < 0.05$ ) NDF omasal flow. There was a tendency for the addition of sucrose to increase ( $P < 0.1$ ) ruminal OMAD and OMTD, while there was no effect ( $P > 0.05$ ) of sucrose addition on intake or digestion of NDF. Concentrate supplementation increased ( $P < 0.001$ ) N intake, flows of N, non-ammonia N, microbial N ( $P < 0.05$ ) and non-ammonia non-microbial N ( $P < 0.01$ ) and apparent total tract digestibility of N ( $P < 0.01$ ) whereas sucrose reduced ( $P < 0.05$ ) N intake and apparent ruminal N digestibility. There was no effect ( $P > 0.05$ ) of either concentrate or sucrose on N use efficiency or efficiency of microbial protein synthesis. Concentrate supplementation increased ( $P < 0.001$ ) plasma  $\beta$  hydroxybutyrate levels.

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In comparison to supplementing unwilted, well-preserved grass silage of moderate digestibility with 3 kg of a starch-based concentrate per day, the limited response to the rate of sucrose supplementation employed suggests that increasing the WSC concentration of grass silage through agronomic and/or ensiling practices will have relatively little effect on intake, rumen digestion or efficiency of microbial N synthesis.

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### **Effect of grass regrowth interval on intake, rumen digestion and nutrient flow to the omasum in beef cattle**

Production and efficient utilisation of high yields of high nutritive value grass throughout the grazing season is critical for cost efficient beef production. Due to environmental legislation and (or) stipulations in environmental schemes, such as the European Union Rural Environmental Protection Scheme, many farmers will now need to operate to lower input criteria than heretofore. The emphasis will be to restrict the application of nitrogen (N) fertilisers and consequently, grassland management systems will reduce in intensity. As livestock production has been identified as a major source of N loss to the environment, it is important to reduce N excretions by improving N utilisation. For the grazing ruminant this inefficiency of N capture is largely due to high concentrations of soluble protein and imbalances in the supply of carbohydrate and protein in the rumen. Nitrogen release into the environment by grazing animals can be diminished by decreasing the crude protein (CP) content of the herbage either through lowering N fertilisation or feeding more mature grass. Research has shown that allowing grass to grow for an extra week (3 vs. 4) resulted in extra herbage production, which was equivalent to the production response to approximately 150 kg fertiliser N ha<sup>-1</sup>. However, as grass matures, the nutritive quality generally declines, due to increased lignification, a decreased proportion of leaves to stem and a decrease in CP content.

The aim of this study was to examine the effects of increasing grass regrowth interval (RI) (28 vs. 38 d) on intake, rumen fermentation, *in situ* degradability, rumen digesta kinetics and nutrient flow to the omasum in beef cattle.

Six ruminally cannulated Holstein-Friesian steers were used in a 2 × 2 crossover design experiment. Digesta kinetics was determined using the rumen evacuation technique and omasal flow was estimated using Co-EDTA, Yb-acetate and indigestible neutral detergent fibre (INDF) as digesta flow markers, and purine bases as microbial markers. Increasing RI had no effect (P>0.05) on dry matter intake, total VFA concentration or molar proportions of VFA in the rumen but reduced (P<0.05) rumen ammonia nitrogen (N) concentrations. Rumen digestion and flow to the omasum of organic matter (OM), neutral detergent fibre (NDF) and N did not differ (P>0.05) between the RI. A higher proportion (P<0.05) of NDF digestion occurred in the rumen with the 28 than the 38d RI. There was no effect (P>0.05) of RI on microbial N flow or on efficiency of microbial nitrogen synthesis but the flow of non-ammonia non-microbial N was reduced (P<0.05) with the 38 than the 28 d RI. The digestion rate (k<sub>d</sub>) of DM, OM and NDF decreased (P<0.05) with increased RI and values were higher with the rumen evacuation than *in situ* incubation method. The passage rate (k<sub>p</sub>) of indigestible NDF was higher than that of the digestible fraction.

These results indicate that increasing the RI of a perennial ryegrass-based sward by 10 days had no adverse effect on feed intake, rumen fermentation or digestion but reduced ammonia N levels in the rumen, potentially reducing nitrogen excretion to the environment.

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### Differentiation of beef according to the pre-slaughter diet of cattle using the stable isotope ratios of carbon and nitrogen

Consumers demand reliable information about the food they buy. In particular, guarantees concerning the authenticity of meats are deemed fundamental to the assurance of food safety, quality and animal welfare. Existing livestock traceability schemes depend ultimately on a paper trail and there is a need for scientific technologies for meat authentication to reassure consumers and to protect regional designations.

Stable isotope (SI) ratio analysis (SIRA) is one potentially useful technique for testing food authenticity. Since dietary C and N stable isotope (SI) compositions (expressed as  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) influence the isotope compositions of these elements in animal tissues,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of animal tissues are useful isotopic markers of diet. The  $\delta^{13}\text{C}$  in livestock animal tissue primarily depends on the proportion of  $\text{C}_3$  and  $\text{C}_4$  photosynthetic plants consumed. The  $\delta^{15}\text{N}$  in such animal tissue is usually less specific for particular dietary inputs but can reflect, among other husbandry practices, the presence of leguminous plants in the diet and also the intensity of agricultural land use in raising the feed crops. The objective of this study was to determine the potential of SIRA to distinguish between beef from cattle that consumed, during the finishing phase, a range of feedstuffs commonly available in Ireland.

In an indoor study, continental cross-bred beef steers ( $n=14/\text{feedstuff}$ ) were offered grass silage, maize silage (cv. Benecia), fermented whole-crop wheat (cv. Soissons), alkalage whole-crop wheat (cv. Soissons) and *ad libitum* concentrates (83% rolled barley). The alkalage was ensiled with 45 kg Home 'N' Dry (Volac International Ltd.)/t dry matter. Forages were offered *ad libitum* through individual Calan gates and supplemented with 3 kg concentrates/head/day. In a grazing study, continental cross-bred steers ( $n=14/\text{feedstuff}$ ) were finished on either a conventionally managed sward i.e. the grass sward received approximately 200 kg N ha<sup>-1</sup>, or an optimally managed grass-clover sward that received 50 kg N ha<sup>-1</sup> in early spring. Animals were slaughtered after approximately 5 months of treatment. After cooling the carcasses for 24 h, *longissimus* muscle was sampled, vacuum packed and stored at -18°C until analysis. Natural abundance isotope ratios of carbon ( $^{13}\text{C}/^{12}\text{C}$ ), and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) were measured on de-fatted muscle tissue by continuous flow isotope ratio mass spectrometry using a Europa Scientific ANCA-NT 20-20 Stable Isotope Analyser and are expressed in delta ( $\delta$ ) notation in parts per thousand. Data were analysed by (Multivariate) Analysis of Variance.

The stable isotope composition of the feeds is shown in Table 41. The  $\delta^{13}\text{C}$  of maize silage was less negative than the other feeds. The  $\delta^{15}\text{N}$  was highest for grass silage and lowest for the concentrate. Muscle SI composition is summarized in Table 42. Feed and muscle  $\delta^{13}\text{C}$  were linearly related: Muscle = 0.41 (feed) - 14.02, se = 0.59,  $P < 0.05$ ,  $R^2 = 0.95$ . Beef from maize silage-fed cattle had the least negative  $\delta^{13}\text{C}$ , reflecting the SI composition of the feed. The  $\delta^{13}\text{C}$  value distinguished ( $P < 0.05$ ) between beef from concentrate/wheat silage, grass silage, grazed grass and grazed grass/clover-fed cattle, but not between beef from alkalage and wheat silage-fed cattle or between beef from alkalage and concentrate-fed cattle. The relationship for  $\delta^{15}\text{N}$  was: Muscle = 0.29 (feed) + 6.36, se = 0.61,  $P = 0.05$ ,  $R^2 = 0.47$ . Using the  $\delta^{15}\text{N}$  value resulted in a poorer discrimination of beef samples compared to using the  $\delta^{13}\text{C}$  value.

A scatter plot of the individual data is shown in Figure 5. Beef from maize silage-fed cattle was clearly distinguished from other samples. The combined isotopic composition of carbon and nitrogen did not improve the discrimination of beef from wheat silage or concentrate-fed cattle beyond that achieved by considering only  $\delta^{13}\text{C}$ .

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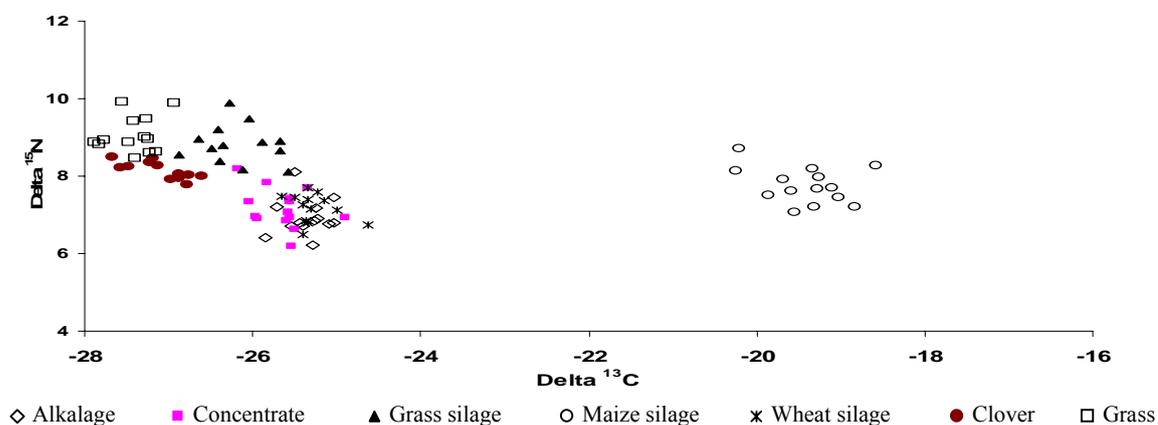
**Table 41: Carbon and nitrogen stable isotope composition of feed ingredients**

Ration	$\Delta^{13}\text{C}$	sd	$\Delta^{15}\text{N}$	sd
Alkalage	-28.20	0.10	4.37	0.73
Concentrate	-27.48	0.30	2.95	0.36
Grass silage	-30.65	0.11	9.31	0.73
Maize silage	-12.74	0.30	6.32	0.26
Wheat silage	-28.02	0.25	3.11	0.36
Grass	-30.37	0.56	5.88	1.14
Grass/clover	-30.24	0.64	4.88	0.97

**Table 42: Carbon and nitrogen stable isotope composition of *Longissimus* muscle from cattle fed various diets pre-slaughter<sup>1</sup>**

Ration	$\Delta^{13}\text{C}$	$\Delta^{15}\text{N}$
Alkalage	-25.36 <sup>b,c</sup>	6.93 <sup>a</sup>
Concentrate	-25.65 <sup>c</sup>	7.16 <sup>a</sup>
Grass silage	-26.18 <sup>d</sup>	8.82 <sup>d</sup>
Maize silage	-19.42 <sup>a</sup>	7.76 <sup>b</sup>
Wheat silage	-25.28 <sup>b</sup>	7.19 <sup>a</sup>
Grass	-27.51 <sup>e</sup>	9.05 <sup>d</sup>
Grass/clover	-27.19 <sup>f</sup>	8.17 <sup>c</sup>
Sed	0.145	0.167
Significance	P<0.001	P<0.001

<sup>1</sup>Within a column means with different superscripts differ (P<0.05)



**Figure 5. Carbon and nitrogen stable isotope ratios in beef from cattle fed different diets.**

It is concluded that differences in the carbon and nitrogen stable isotope composition of the feeds examined were reflected in the muscle of cattle thereby allowing SIRA to be used as a

component, at least, of a scheme to authenticate the dietary history of beef from cattle consuming these feeds.

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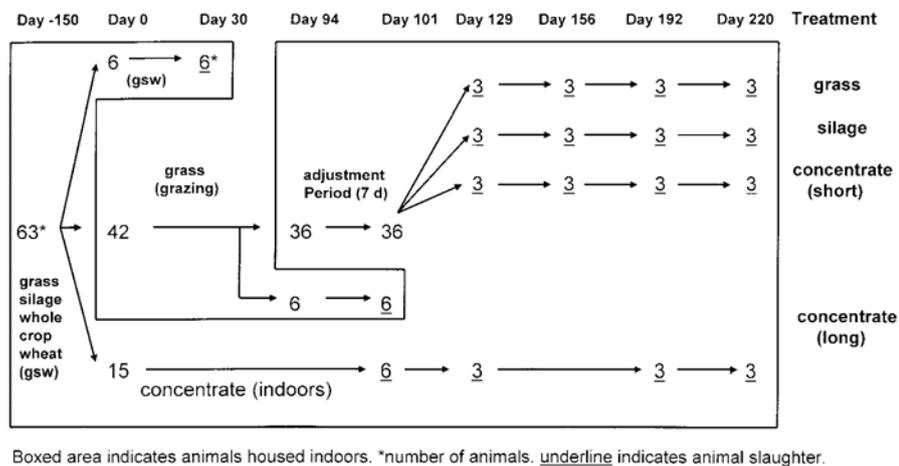
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**Temporal change in the carbon stable isotope ratios of beef following a change in ration composition**

Information concerning the production and origin of foods of animal origin is increasingly sought by consumers. In particular, guarantees of food authenticity and traceability, are required. There is, therefore, a need to develop methodologies capable of providing information about the origin and background history of animal-derived foods. On-going research aims to establish whether the isotopic composition of light elements ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta\text{D}$ ,  $\delta^{18}\text{O}$  and  $\delta^{34}\text{S}$ ) can be used as an intrinsic, biochemical marker for tracing and authenticating beef. The specific objectives of this experiment were 1) to determine the temporal variability in the stable isotope composition of C ( $\delta^{13}\text{C}$ ) in feed materials over one growing season, and 2) to determine the speed at which changes in the isotopic composition of beef muscle tissue occur following a change in diet.

From a group of 63 heifers, 15 were fed concentrates for 220 days and 36 were grazed for 97 days, then housed, and offered in groups of 12 one of three diets differing in the proportion of concentrates, silage and grass (Figure 6). Heifers were slaughtered at approximately monthly intervals (n=3 per date per diet) and feed samples were collected regularly from June to November. Two control groups (n=6) were slaughtered at day 30 and day 101. Samples of the strip loin (*M. longissimus dorsi*) were collected, freeze-dried and milled. The  $\delta^{13}\text{C}$  of bulk muscle and bulk feed materials was determined by Isotope Ratio Mass Spectrometry.



**Figure 6. Experimental design.**

The isotopic composition of the concentrate and grass silage showed little temporal variation (range  $<0.6\text{‰}$   $\delta^{13}\text{C}$ ) over a 5-month period. Grass, by contrast, showed marked temporal trends over this period with monthly values ranging from  $-29.6$  to  $-31.0\text{‰}$  in  $\delta^{13}\text{C}$ . There was an isotopic difference (about  $3\text{‰}$   $\delta^{13}\text{C}$ ) between concentrate and grass/silage feed materials (Figure 7).

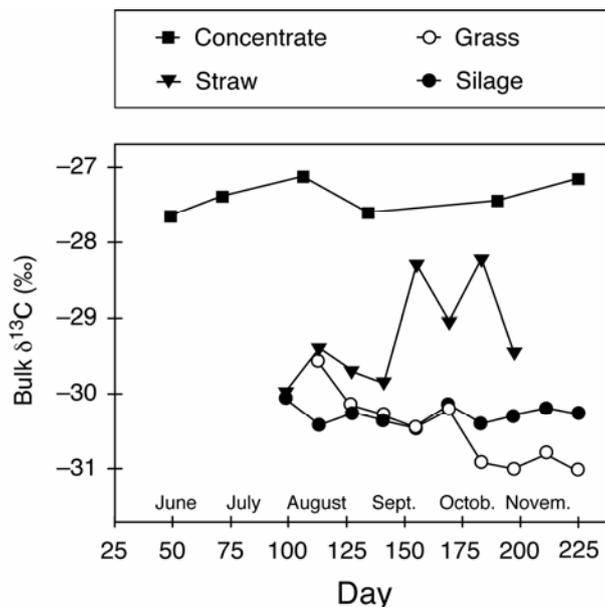


Figure 7. Isotopic composition of feed materials.

The isotopic composition of muscle tissue changed slowly after diets were switched. However, even small isotopic differences between grass- and concentrate-based diets were consistently reflected in bulk muscle tissue (Figure 8). The final difference in muscle  $\delta^{13}\text{C}$  between the grass and ‘long concentrate’ treatments was 1.9‰.

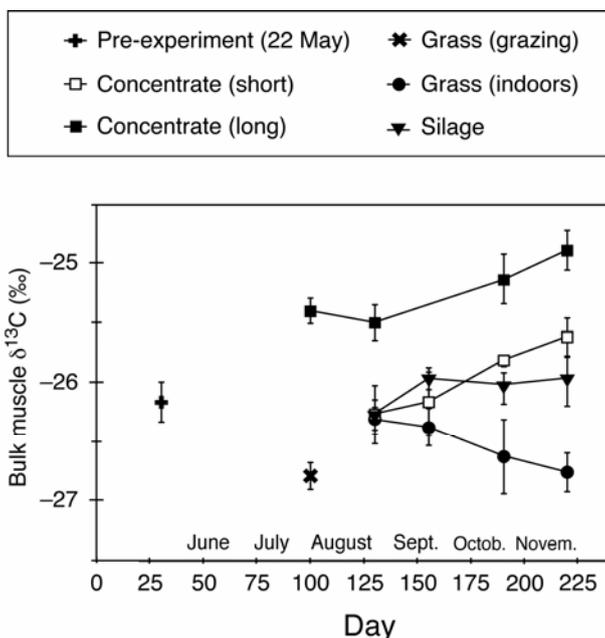


Figure 8. Isotopic composition of muscle tissue.

It is concluded that (1) while  $\delta^{13}\text{C}$  showed little temporal variation in concentrate and silage feed materials, it was more variable in fresh grass over one growing season, and (2) bulk muscle tissue reflected small isotopic differences between dietary components after about 100 days.

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## Hooves: a new tissue for high-resolution reconstruction of bovine dietary histories

Stable isotope analysis of incremental tissues such as hair and teeth are powerful tools used to track dietary changes and movement in animals. Spatially separated samples record the isotopic composition of the tissue at the time it was deposited. Our objective was to establish whether sequential analysis of hooves can be used to reconstruct the dietary history of cattle.

A controlled, on-farm, experiment was conducted in which six cattle were switched from a barley-based diet to an isotopically distinct diet incorporating maize and urea (the isotopic spacing between diets was 15‰ for  $\delta^{13}\text{C}$  and 11‰ for  $\delta^{15}\text{N}$ ) and maintained on that diet for 168 days. Postmortem sampling of the cleaned wall of the outer, left front toe was carried out using a micro-drilling technique. A 15 mm thick slice of the toe was cut with a band saw, 15 mm away from the inner wall. The soft tissues were removed with a blade and the horn defatted and dried. Bands less than 1 mm deep were drilled into the hoof wall using a diamond drill bit attached to a Dremel<sup>®</sup> 400 drill. The average width of sampled bands was 1.2 mm and the spacing between them was less than 1 mm. Therefore, at least 25 samples with a mean C and N content of  $393 \pm 46 \mu\text{g}$  and  $116 \pm 19 \mu\text{g}$  ( $n=198$ ,  $\pm\text{SD}$ ) were collected from the top 60 mm of each toe.

The isotopic composition of hooves responded very quickly to the new diet, suggesting that at least one of its pools has a rapid turnover with a half-life of less than 20 days. However, the N response was delayed somewhat compared to that of C. The calculated mean growth rate of cattle hooves was  $6.85 \pm 0.79$  mm per month ( $n=6$ ,  $\pm\text{SD}$ ), a value that is considerably higher than previous estimates. The temporal resolution of sampling used here was about 5 days.

In conclusion, these experimental results demonstrate for the first time that hooves are a suitable incremental tissue for high-resolution isotopic reconstruction of the dietary and life history of bovine animals.

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## How fast are ratio dietary carbon and nitrogen replenished in bovine muscles?

Stable isotope analysis of animal tissue has potential to authenticate the dietary history of meat animals. Stable isotope (SI) ratio analysis (SIRA) was used to investigate the turnover of carbon and nitrogen in bovine *Longissimus dorsi* and *Psoas major* muscles. 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  $^{15}\text{N}$  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 *L. dorsi* and *P. major* muscles were collected at 24 h post-mortem and processed (de-fatted) for SIRA. Isotopic equilibrium was not reached in either tissue for  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  after 168 days of feeding the

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isotopic diet. The slow turnover of C and N was reflected in half-lives of 151 and 157 days for *L. dorsi* and 134 and 145 days for *P. major*, respectively. It is concluded that bovine *L. dorsi* and *P. major* tissues have similar slow turnover rates of C and N which has implications for authenticating dietary history of cattle on a long term basis.

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### Experimental determination of turnover of carbon isotopes in bovine hair and hoof

Stable isotopes measured in continually growing keratinised tissues like hair or hoof have proven to be a useful tool for reconstructing the individual history at a weekly to daily precision for 1 to 2 years prior to sampling date. Quantitative reconstruction of dietary preference requires a precise estimate of tissue turnover by means of controlled feeding experiments. However, very limited data are available so far, and turnover rates have only been estimated for two mammal species, mice and horses. In this study, we determined the turnover rates of carbon in hoof and tail hair of growing steers fed a C<sub>3</sub>, barley-based diet followed by a C<sub>4</sub>, maize-based diet 168d prior slaughter. It was found that turnover in steer hair could be described by a three-pool modelling approach as previously proposed by Ayliffe et al. (2004). The carbon isotope record of the diet change was consistent with a pool having a fast turnover rate ( $t_{1/2}$ ~1.7 days) that made up ~53% of the isotope signal, a pool with an intermediate turnover rate ( $t_{1/2}$ ~7.7 days) that comprised ~20% of the isotope signal, and a pool with slow turnover rate ( $t_{1/2}$ ~69.1 days) that made up ~28% of the total isotope signal. Two pools only could be identified in steer hoof, with a pool having an intermediate turnover rate ( $t_{1/2}$ ~11.7 days) that made up ~52% of the isotope signal, and a pool with a slower turnover rate ( $t_{1/2}$ ~34.0 days) that comprised 45% of the isotope signal. Hoof responded more slowly than hair from the same individual to the diet-switch and the amplitude of short-term isotope changes was reduced. This result is interpreted as a reflection of time-averaging due to cross-sampling of histological features in hoof. Using the model parameters determined for steer hair an estimate with an error of 1‰ or less, the  $\delta^{13}\text{C}$  value of the steer diet during our experiment (including an unplanned dietary switch) was made. Comparison of model parameters calculated for horses and steers suggest that the half-life of the fast responding pool is controlled by passage rates, whereas the size of this reservoir seems mainly controlled by the protein content of the diet. These findings are in agreement with the interpretation of the fast turnover reservoir reflecting exogenous (dietary) carbon whereas the other two could correspond to recycling of endogenous tissues via body tissue turnover. Our results suggest that the 3-pool model probably applies to all mammals, with small adjustments in the size and rate constants of individual reservoirs.

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### Supplementation of heifers with ruminally-protected polyunsaturated fatty acids (PUFA): effects on colour stability of retail-packaged minced beef

Meat and milk of ruminant origin contain a substantial proportion of saturated lipids which result from lipolysis and biohydrogenation of dietary unsaturated lipids by rumen

microorganisms. If a more unsaturated lipid composition is desired, rumen hydrogenation must be prevented. Strategies to achieve this usually involve chemical modification of dietary lipids or feeding a seed source of dietary lipids where the oilseed itself provides some degree of protection from rumen microbial activity. Dietary PUFA are preferentially deposited in membrane phospholipids in ruminants and the fatty acid composition of phospholipids has been shown to be largely responsible for the susceptibility of meat to lipid oxidation. Therefore, supplementation of cattle with ruminally-protected polyunsaturated fatty acids (RP-PUFA) and a consequent increase in PUFA in muscle lipids, would be expected to decrease the colour stability of the meat.

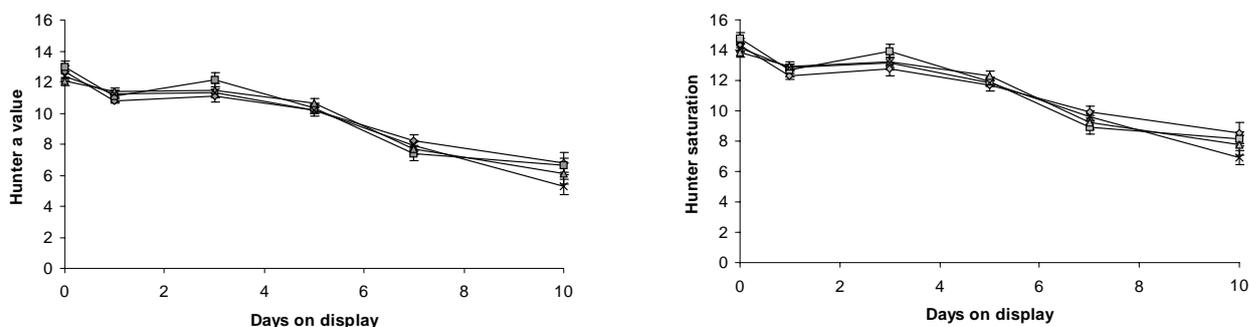
Additionally, mincing of beef destroys cellular compartmentalisation and, thus, brings pro-oxidant components into close proximity with labile PUFA. There is also evidence that packaging of meat in a high oxygen atmosphere typical of beef retail packs provides an additional challenge to the oxidative stability and, thus, colour stability of meat, particularly if mincing has occurred. The objective of the present study was to determine the effect of supplementation of heifers with a source of long chain  $\omega$ -3 RP-PUFA on the colour stability of minced beef, hypothesising that such supplementation would decrease the colour stability of beef under simulated retail display conditions.

Continental crossbred heifers were individually offered a daily bolus ration of 1kg (freshweight, approximately 850g dry matter (DM)) that contained the  $\omega$ -3 RP-PUFA supplement of eicosapentaenoic acid (EPA, C20:5 $\omega$ -3) and docosahexaenoic acid (DHA, C22:6 $\omega$ -3) (Nutreco Holding N.V., 3800 AG Amersfoort, The Netherlands) at 0 (control), 69, 138 or 275g PUFA per kg (PU00, PU69, PU138 and PU275, respectively). This was followed by 1.5kg (1.28kgDM) of a high crude protein (26.2g/100g) balancer ration. Each afternoon 3.5kg (3.0kgDM) of another balancer ration was offered to all heifers with 1.5kg (1.24kgDM) of straw. Diets were formulated to be isoenergetic, isolipidic and isonitrogenous. Vitamin E, as dl- $\alpha$ -tocopheryl acetate was added to the RP-PUFA supplement at a rate of 5,000mg/kg and to the vitamin/mineral supplement included in the balancer rations at a rate of 10,000 I.U./kg (1mg dl- $\alpha$ -tocopheryl acetate = 1 I.U. (international units)). After 8 weeks of supplementation, heifers were humanely harvested, samples of neck muscle were recovered and were stored at -80°C (6 months) prior to mincing. Eighteen hours before mincing samples were placed in a dark chill room at <2°C to thaw. Sections of neck muscle were minced by passing through a mincer with 3mm holes. When minced, samples were divided into six approximately equal portions, formed into patties of least 2.54cm thickness and dispensed into styrofoam trays with absorbent pads. Trays were sealed under oxygen impermeable barrier film (oxygen transmission rate: 8cm<sup>3</sup>O<sub>2</sub>/m<sup>2</sup>/24h at 23°C and 75% relative humidity) following evacuation and flushing with 80%O<sub>2</sub>:20%CO<sub>2</sub> in a modified atmosphere packaging machine. Trays were randomly positioned in an open-fronted retail display cabinet under permanent fluorescent lighting (2800 lm) and permanently shielded by an insulating blind, to maintain a uniform temperature distribution. Cabinet temperature was monitored using three needle thermocouples.

Approximately 3 hours after packaging, the 'L' (lightness), 'a' (redness) and 'b' (yellowness) values of the minced beef were measured (day 0) using a benchtop Hunter lab UltraScan XE spectrophotometer. Saturation and hue angle were calculated as  $\sqrt{a^2+b^2}$  and  $[\tan^{-1}(b/a)][180/\pi]$ , respectively. Colour was measured again on days 1, 3, 5, 7 and 10. Data were analysed using ANOVA appropriate to a split-plot design. Treatment (4 levels) was in the main plot and display time (6 levels) in sub-plot.

There was no effect of treatment or treatment  $\times$  display time interaction for any of the measured colour variables except for 'b' value (treatment  $\times$  display time,  $P = 0.038$ ). Thus while the PU00 'b' value tended to be lowest up to day 5, it was equal to PU275 on day 7 but higher ( $P < 0.05$ ) on day 10. Between days 7 and 10, the PU00 'b' value tended to decrease

( $\Delta = -0.46$  b units,  $P > 0.05$ ), whereas the PU275 'b' value decreased ( $P < 0.05$ ) by 1.04 b units. This may prove useful in providing an index of colour stability for display periods in excess of 7 days but future trials are required to reinforce this suggestion. Redness and saturation are normally used as indices of colour stability (Figure 9), which are responsible for the phenomenon seen as unstable surface colour of meat, as red oxymyoglobin is converted to brown metmyoglobin. Critical considerations in the present study may be the muscle concentration of vitamin E as well as PUFA and also the low mean cabinet temperature ( $2.6^{\circ}\text{C}$ ).



**Figure 9. Redness (Hunter 'a' value) and saturation of minced beef from heifers offered ruminally-protected PUFA at 0 (PU00 (control)  $\diamond$ ), 69 (PU69,  $\square$ ), 138 (PU138,  $\triangle$ ) or 275 (PU275,  $\times$ ) g/day and packaged in 80%O<sub>2</sub>:20%CO<sub>2</sub> under permanent fluorescent lightning (2800lm).**

It is concluded that supplementing heifers with RP-PUFA did not have a deleterious effect on colour stability of minced beef stored for 1 week in high O<sub>2</sub> packs relative to control heifers, when indicated by redness or saturation.

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### Feeding a diet with CLA-enriched beef improves 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 fishoil) 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 three dietary treatments as stated in Table 43 for a 28 day period. Relative to a low-CLA beef-based ration, both the high-CLA beef and synthetic CLA diets significantly reduced serum glucose, TAG ( $p < 0.05$ ) and NEFA ( $p < 0.05$ ) concentrations. The high CLA

diets also significantly reduced plasma IL-6 ( $p < 0.05$ ) levels and increased plasma adiponectin and cholesterol ( $P < 0.05$ ) concentrations ( $p < 0.05$ ) compared to controls.

**Table 43: Whole Body Metabolic Data – Mean (SEM)**

	Low-CLA Diet (n=7)	Beef	High-CLA Diet (n=8)	Beef	Low-CLA beef + Synthetic CLA Diet (n=7)
Glucose (mmol/l)	16.63 (0.76)		14.07 (1.04)*		15.28 (1.48)*
Cholesterol (mmol/L)	5.14 (0.42)		5.83 (0.17)*		5.86 (0.46)*
Triglycerides (mmol/L)	1.49 (0.06)		1.38 (0.05)*		1.36 (0.04)*
NEFA (mmol/L) <sup>2</sup>	0.70 (0.07)		0.36 (0.07)*		0.45 (0.06)*
IL-6 (pg/ml)	1626.33 (138)		1167.67 (168)*		1151.24 (109)*
Adiponectin (mg/ml)	7198.2 (635)		9961.05 (226)*		9211.94 (706)*

\* $P < 0.05$  Value denotes significant differences compared to the control group (Low-CLA Beef Diet). <sup>1</sup>Produced from cattle fed a high concentrate ration. <sup>2</sup>Non-esterified fatty acids.

The preliminary 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. Additional analysis is required to determine the molecular basis of these effects.

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### The record of dietary change and climatic conditions in 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 the balance between tissue turn-over and tissue growth and (c) to determine if climatically driven O and H (possibly S) and geologically driven Sr isotope differences within Ireland are large enough to distinguish between animals raised in different parts of the country. Three experiments are in progress to address these objectives.

**Experiment 1:** 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. Lambs were offered one of two dietary allowances, to achieve two target growth rates. 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. The experiment is in progress.

**Experiment 2:** This experiment aims to investigate the record of long-term and short-term dietary shifts in incremental tissues, focussing mainly on teeth. Because permanent cheek teeth (molars and premolars) are mostly formed during the second year of the animal's life, this experiment is being carried on 1 year-old sheep. Fifteen one year-old sheep, previously

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at pasture were brought to Grange. One group (n=3) was slaughtered at the beginning of the study while the remainder were offered an isotopically distinct ration for 2, 4, 8 weeks prior to return to a grass silage ration, or continually. Breath, wool and blood is collected periodically from selected animals and post-slaughter, teeth, hooves and wool will be sampled.

**Experiment 3:** Several sites were to be chosen across Ireland. The criteria for selection were: the locations must represent inland and coastal, as well as lowland and hilly areas, in order to sample the isotope variability in local water and feeding resources within the country and; sheep should be raised under extensive conditions and they should be fed only with local resources. On each site, three lambs of the same breed were chosen. These animals stay in their flock and go to the pastures without receiving any special treatments. At the beginning of the experiment an area of 5 cm x 5 cm located on the animal's back was shaved and the hair discarded. The newly grown wool from the same area of the skin is sampled at three-monthly intervals and kept for analysis.

*RMIS No. 5214 & 5430*

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### **Accumulation of intermediates during *in vitro* ruminal fermentation of polyunsaturated plant oils and oilseeds**

Food products from ruminants are the major sources of conjugated linoleic acids (CLA) for humans. CLA in ruminant meat originates from ruminal biohydrogenation of dietary linoleic acid (C18:2 n-6) and from ruminally derived vaccenic acid (VA) that is desaturated in the tissue by delta-9 desaturase. The objective of this experiment was to measure the accumulation of intermediates during biohydrogenation of two sources of polyunsaturated fatty acids, 1) camelina, a rich source of both linoleic and linolenic acid (C18:3 n-3) and 2) linseed a rich source of linolenic acid, in both oil and seed form, using a ruminal simulation system.

Five rations each formulated to contain 60g lipid/kg of dry matter were examined. The main lipid sources were; 1) megalac (MG), (control treatment), 2) camelina oil (CO), 3) linseed oil (LO), 4) camelina seed/NaOH treated (CS), and 5) linseed/NaOH treated (LS). CS and LS were ground using a Vita-mix 3600 set at speed 4 (20 seconds CS) and (30 seconds LS) to give CSG and LSG respectively, to simulate mastication. Each treatment was incubated, in duplicate in rumen fluid from six cannulated ewes, for 0, 3, 6, 10, 16 and 24 hours at 39°C. Fatty acids were extracted using chloroform methanol (C/M, 2/1,v/v), methylated and fatty acid methyl esters (FAME) were measured by gas chromatography using a CP-Sil 88 column. Data were subjected to ANOVA and all pair-wise comparisons between means were carried out using Tukey's test.

The mean fatty acid proportions (in total lipid) after 6, 10, and 24 hours incubation are summarised in Table 44. Camelina and linseed resulted in greater proportions of C18:2n6 and C18:3n3 than MG. After 24 hours incubation the proportion of C18:2 n-6 was greatest for LS, but not different from CS or LSG. The proportion of C18:3 n-3 was also greatest with LS at 24 hours incubation but not different from LSG. Supplying the fat source as oil compared to seed form generally resulted in a lower proportion of CLA cis-9, trans-11. However, the difference in proportions of CLA cis-9, trans-11 between all treatments at 24 hours incubation was non-significant. Alternatively higher proportions of VA accumulated when using oils as opposed to seeds. Grinding of the whole-seeds resulted in an increase in the proportion of VA

for CSG compared to CS and LS. The proportion of VA for LSG was higher but not different from LS.

The greater accumulation of linoleic acid and linolenic acid for both CS and LS suggests that oil seeds were more effective in reducing the rate of ruminal hydrogenation than oils. The lower proportions of linoleic and linolenic acid and higher proportions of vaccenic acid present in both CSG and LSG compared to CS and LS suggests that processing the seed prior to incubation increased the availability of polyunsaturated fatty acids for ruminal hydrogenation.

**Table 44: The effect of treatment and incubation time on fatty acid composition (g/100g of fatty acid)**

Treatment	MG	CO	LO	CS	LS	CSG	LSG	SED	sig
<i>6 hours</i>									
C18:2n6	11.01 <sup>c</sup>	16.70 <sup>b</sup>	17.50 <sup>ab</sup>	16.60 <sup>b</sup>	18.01 <sup>a</sup>	16.20 <sup>b</sup>	17.14 <sup>ab</sup>	0.43	***
C18:3n3	1.10 <sup>e</sup>	26.20 <sup>cd</sup>	41.70 <sup>b</sup>	30.41 <sup>c</sup>	48.25 <sup>a</sup>	24.10 <sup>d</sup>	51.05 <sup>a</sup>	1.48	***
CLA 9,11	0.04 <sup>b</sup>	0.40 <sup>ab</sup>	0.54 <sup>ab</sup>	0.16 <sup>b</sup>	0.40 <sup>ab</sup>	0.26 <sup>ab</sup>	0.92 <sup>a</sup>	0.24	**
VA	2.40 <sup>ab</sup>	2.42 <sup>ab</sup>	2.24 <sup>b</sup>	1.98 <sup>b</sup>	1.25 <sup>b</sup>	4.15 <sup>a</sup>	0.91 <sup>b</sup>	0.59	***
<i>16 hours</i>									
C18:2n6	10.02 <sup>d</sup>	13.63 <sup>c</sup>	14.55 <sup>bc</sup>	14.83 <sup>bc</sup>	17.33 <sup>a</sup>	12.88 <sup>c</sup>	16.32 <sup>ab</sup>	0.64	***
C18:3n3	1.08 <sup>e</sup>	19.82 <sup>d</sup>	31.48 <sup>c</sup>	26.38 <sup>cd</sup>	49.71 <sup>a</sup>	18.99 <sup>d</sup>	41.23 <sup>b</sup>	2.55	***
CLA9,11	0.01 <sup>b</sup>	0.33 <sup>b</sup>	0.61 <sup>b</sup>	0.48 <sup>b</sup>	0.67 <sup>b</sup>	0.60 <sup>b</sup>	1.55 <sup>a</sup>	0.28	***
VA	3.99 <sup>bc</sup>	5.36 <sup>ab</sup>	6.16 <sup>a</sup>	3.98 <sup>bc</sup>	1.13 <sup>d</sup>	6.56 <sup>a</sup>	2.02 <sup>cd</sup>	0.7	***
<i>24 hours</i>									
C18:2n6	7.84 <sup>b</sup>	6.29 <sup>b</sup>	8.31 <sup>b</sup>	11.76 <sup>a</sup>	14.08 <sup>a</sup>	6.24 <sup>b</sup>	13.04 <sup>a</sup>	1.06	***
C18:3n3	1.91 <sup>d</sup>	5.84 <sup>cd</sup>	12.71 <sup>bc</sup>	16.92 <sup>b</sup>	43.41 <sup>a</sup>	7.71 <sup>bcd</sup>	38.62 <sup>a</sup>	3.1	***
CLA9, 11	0.04 <sup>a</sup>	0.45 <sup>a</sup>	0.42 <sup>a</sup>	0.63 <sup>a</sup>	0.63 <sup>a</sup>	0.68 <sup>a</sup>	0.33 <sup>a</sup>	0.25	ns
VA	5.19 <sup>b</sup>	11.21 <sup>a</sup>	13.1 <sup>a</sup>	4.77 <sup>b</sup>	2.61 <sup>b</sup>	14.14 <sup>a</sup>	3.75 <sup>b</sup>	1.48	***

ns = non-significant; \*\*\*<0.001; \*\*<0.01.

Values not sharing a common superscript are significantly different.

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### Accumulation of biohydrogenation intermediates during *in vitro* ruminal fermentation of camelina oil-based rations

The myriad putative health benefits of conjugated linoleic acid (CLA) and in particular the cis-9, trans-11 isomer, have stimulated interest in increasing its concentration in food. Ruminant fat is the main dietary source of CLA for humans and CLA is produced in the rumen by incomplete biohydrogenation of dietary linoleic acid (LA). It is now accepted that most CLA is synthesised post-ruinally by desaturation of vaccenic acid (VA) produced during ruminal biohydrogenation of (LA) and linolenic acid (LNA). Enhancement of VA synthesis in the rumen is, therefore, an important element of strategies to increase CLA concentration in tissue. The objective of this experiment was to determine the effect of controlling the rate of release of oil from camelina seeds, a novel source of both LA and LNA, on the accumulation of intermediates during ruminal biohydrogenation.

Five rations formulated to contain 60g lipid/kg of dry matter were examined. The main lipid sources were; 1) megalac (MG), (control), 2) camelina oil (CO), 3) camelina seed (CS); (treated with 100g NaOH/kg seed), 4) camelina amide (CA); (CO reacted with 0.73g ethanolamine/g oil) and 5) Ground CS (CSG), (ground in a Vita Mix 3600, set at speed 4 for 20 seconds per 100g, to simulate mastication). Samples were incubated (in duplicate) in

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rumen fluid from six cannulated ewes for 0,3,6,10,16 and 24 hours at 39°C in three separate runs. Lipids were extracted using chloroform methanol (C/M, 2/1, v/v), dried under nitrogen, methylated and fatty acid methyl esters (FAME) were analysed by gas chromatography. Data were subjected to ANOVA and all pair-wise comparisons were carried out using Tukey's t-test.

Fatty acid proportions (in total lipid) after 3, 6 and 10 hours incubation are summarised in Table 45. CA caused the greatest increase in CLA trans-10, cis-12, CLA cis-9, trans-11 and VA concentrations up to six hours and VA concentrations over all time-points shown. VA concentrations were lowest for CS at all time-points reported. On average, accumulation of CLA and VA was similar ( $P>0.05$ ) for the oil and whole seeds and for whole and ground seeds.

**Table 45: Effect of ration and incubation time on fatty acid composition (g/kg)**

Treatment	MG	CO	CS	CA	CSG	SED	Sig.
<i>3 Hours</i>							
C18:2n6	130.4 <sup>c</sup>	175.5 <sup>b</sup>	170.6 <sup>b</sup>	220.3 <sup>a</sup>	169.1 <sup>b</sup>	11.2	***
C18:3n3	78.8 <sup>b</sup>	286.8 <sup>a</sup>	331.9 <sup>a</sup>	132.3 <sup>b</sup>	297.6 <sup>a</sup>	43.3	***
CLA 10,12	0.70 <sup>b</sup>	0.10 <sup>b</sup>	0.10 <sup>b</sup>	2.20 <sup>a</sup>	0.70 <sup>b</sup>	0.30	***
CLA 9,11	0.80 <sup>b</sup>	1.00 <sup>b</sup>	1.10 <sup>b</sup>	7.80 <sup>b</sup>	0.80 <sup>b</sup>	1.60	***
VA	1.28 <sup>b</sup>	1.14 <sup>b</sup>	1.13 <sup>b</sup>	4.01 <sup>a</sup>	1.84 <sup>ab</sup>	0.89	*
<i>6 Hours</i>							
C18:2n6	112.9 <sup>c</sup>	168.8 <sup>b</sup>	164.0 <sup>b</sup>	202.0 <sup>a</sup>	163.4 <sup>b</sup>	10.8	***
C18:3n3	1.14 <sup>d</sup>	26.85 <sup>b</sup>	31.80 <sup>a</sup>	10.70 <sup>c</sup>	27.82 <sup>b</sup>	1.19	***
CLA 10,12	0.40 <sup>b</sup>	0.20 <sup>b</sup>	0.50 <sup>b</sup>	2.10 <sup>a</sup>	0.60 <sup>b</sup>	0.20	***
CLA 9, 11	0.20 <sup>b</sup>	1.20 <sup>b</sup>	2.40 <sup>b</sup>	7.20 <sup>a</sup>	3.00 <sup>ab</sup>	1.50	***
VA	22.2 <sup>b</sup>	24.4 <sup>ab</sup>	18.0 <sup>b</sup>	66.4 <sup>a</sup>	24.5 <sup>ab</sup>	14.9	*
<i>10 Hours</i>							
C18:2n6	101.7 <sup>b</sup>	138.1 <sup>ab</sup>	148.1 <sup>a</sup>	158.9 <sup>a</sup>	135.2 <sup>ab</sup>	12.8	**
C18:3n3	10.5 <sup>d</sup>	206.6 <sup>b</sup>	275.1 <sup>a</sup>	72.2 <sup>c</sup>	197.0 <sup>b</sup>	16.8	***
CLA 10,12	0.60 <sup>b</sup>	0.30 <sup>b</sup>	5.10 <sup>b</sup>	3.70 <sup>a</sup>	0.70 <sup>b</sup>	0.40	***
CLA 9, 11	0.10 <sup>b</sup>	2.90 <sup>ab</sup>	9.40 <sup>ab</sup>	3.40 <sup>ab</sup>	6.80 <sup>a</sup>	1.50	**
VA	36.0 <sup>b</sup>	48.8 <sup>b</sup>	35.8 <sup>b</sup>	98.9 <sup>a</sup>	56.9 <sup>ab</sup>	16.3	**

CLA 9,11 = CLA cis-9, trans-11; CLA 10,12 = CLA cis-10, trans-12.

\*\*\*<0.001; \*\*<0.01; \*<0.05. Within a row, means not sharing a common superscript are significantly different.

Altering the rate of release of camelina oil to rumen fluid *in vitro* influenced the pattern of dietary fatty acid metabolism. Chemical protection of oil with ethanolamine was most effective in increasing the accumulation of VA suggesting that it was not effective in reducing the rate of biohydrogenation. Accumulation of VA for CS tended to be lower compared to CO indicating that oilseeds offered more protection than oils to ruminal biohydrogenation. The greater accumulation of VA for CSG compared to CS suggests that processing the seed prior to incubation increased the rate of biohydrogenation compared to the unprocessed seed.

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