

MEAT QUALITY

Long chain polyunsaturated fatty acids in muscle from heifers offered increasing levels of a supplement containing ruminally-protected eicosapentaenoic and docosahexaenoic acid

Polyunsaturated fatty acids (PUFA), particularly the long chain PUFA (LC-PUFA) eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) are recognised as being of fundamental importance to human health. Although meat from grass-fed ruminants contains moderate levels of these n-3 (omega-3) LC-PUFA, their concentration is constrained by ruminal biohydrogenation. In order to elevate concentrations in edible ruminant tissues, strategies have been developed to prevent or reduce the susceptibility of these LC-PUFA to ruminal biohydrogenation. The objective of this study was to determine the effectiveness of supplementing heifers with increasing amounts of ruminally-protected (RP) n-3 LC-PUFA on muscle concentrations of EPA and DHA.

Continental crossbred heifers (n = 48) were assigned to treatment in a completely randomised design. Each morning, heifers were offered a bolus ration of 1kg (freshweight, approximately 850g dry matter (DM)) that contained the proprietary RP omega-3 PUFA supplement of EPA and DHA. The bolus ration was formulated to contain 0 (control), 69, 138 and 275g LC-PUFA per kg (PU00, PU69, PU138 and PU275, respectively) and heifers were fed their respective rations individually using Calan Broadbent feeders. Diets were formulated to be isoenergetic, isolipidic and isonitrogenous. After slaughter in a commercial facility, neck muscle samples were stored at -30°C under vacuum prior to analysis. Fatty acids were extracted from muscle tissue, separated into neutral (NL) and polar (PL) lipids, derivatised and gas chromatographic analysis of fatty acid methyl esters (FAME) was performed. Data were analysed using PROC GLM and the response to incremental dietary LC-PUFA supplementation was tested using orthogonal polynomial contrasts (CONTRAST statement, SAS). Data for EPA and DHA in NL were found not to be normally distributed and were analysed using a non-parametric Kruskal-Wallis ANOVA. EPA, expressed as a proportion of total FAME, in PL increased linearly ($P < 0.001$) with increasing dietary amounts of RP n-3 LC-PUFA, while there was an overall treatment effect ($P = 0.0433$) on DHA in PL, which increased quadratically ($P = 0.0281$) (Figure 13). In NL, there was an effect of treatment on both EPA ($P = 0.002$) and DHA ($P < 0.001$) proportions, with PU275 having higher proportions of both fatty acids, but no clear pattern was evident

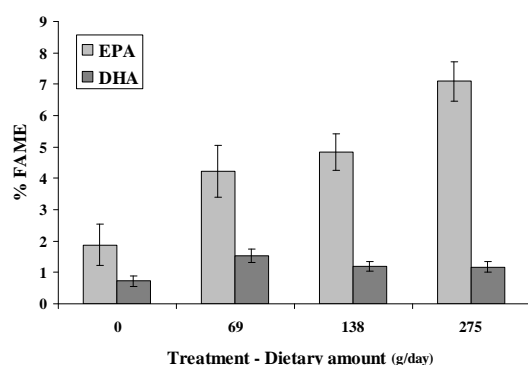


Figure 13. Response of EPA and DHA (as a proportion of total FAME) in muscle phospholipids to increasing dietary inclusion of RP n-3 LC-PUFA.

It is concluded that provision of increasing amounts of a dietary RP n-3 LC-PUFA supplement to heifers was effective at increasing proportions of both EPA and DHA in polar lipids of muscle.

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Moloney, A.P., Dunne, P.G.¹, Childs, S.², Monahan, F.J.² and Kenny, D.A.²

¹Teagasc, Ashtown Food Research Centre, Dublin

²UCD School of Agriculture, Food Science and Veterinary Medicine, Belfield, Dublin

The digestibility of intact sunflower seeds in beef cattle

The putative human health benefits of conjugated linoleic acid (CLA) have focussed attention on increasing its concentration in food. Ruminant fat is the major “natural” source of CLA which is formed in part, by isomerisation and biohydrogenation of dietary linoleic acid (LA) in the rumen. Thus, feeding a rich source of LA such as sunflower oil can increase the CLA concentration in muscle. In production systems where concentrates are offered in discrete meal(s), separately to forage, maintaining a high amount of oil provides technical challenges requiring absorbent co-ingredients and modified management of the feeds to prevent expulsion of the oil. Feeding whole sunflower seeds is one possible solution to this problem. However, there is little information available as to whether sunflower seeds require some pre-treatment to facilitate digestion. The objective of this study was to determine the comparative digestibility of sunflower oil and two varieties of sunflower seeds in beef cattle.

Two varieties of sunflower seeds (*Helianthus annuus*) were examined, the black/high-oil and the confectionary (striped)/lower-oil seed varieties. Based on the measured oil concentration (469 and 309 g/kg for the black and confectionary seeds, respectively), sufficient seeds were added to a wheat pollard based ration to supply 135 g oil/kg concentrate. A sunflower oil based ration was also formulated to a similar oil concentration. Twenty four Friesian steers (252 s.d. 21.3 kg) were assigned at random to one of two groups of 12 animals each to facilitate measurement of *in-vivo* digestibility. Within each group, animals were individually offered at random, one of the three experimental concentrates or a barley/soyabean control ration (target = 6g/kg body weight) separately from grass silage (target = 13 g dry matter (DM) / kg bodyweight). Each group of animals was offered their respective rations for 16 days followed by total collection of faeces for eight days. Representative subsamples of feed and faeces from each animal were chemically analysed. Representative samples of faeces were also washed over a sieve of 2mm aperture to recover intact seeds. Data were subjected to analysis of variance in Genstat, using a model that had group and treatment as main effects.

The target ration consumption was achieved (Table 44). Oil represented 67, 60, 76 and 30g/kg DM consumed for the striped seeds, black seeds, sunflower oil and control rations, respectively. The digestibility of DM and organic matter (OM) was higher ($P<0.05$) for the control ration compared to the sunflower-based rations. Within the latter, the digestibility of DM, OM, ash and neutral detergent fibre was lower ($P<0.05$) for the striped seeds ration compared to the black seeds or free oil rations. The digestibility of oil in the free oil ration was similar to that in the striped seeds ration but higher ($P<0.05$) than that in the black seeds rations. The digestibility of oil and seeds was similar for both seed types.

Table 44: Dry matter intake (DMI) and *in vivo* digestibility of sunflower seeds and oil

	Striped seeds	Black seeds	Sunflower oil	Control	Sed	Significance
Silage DMI						
(kg/d)	3.20	3.09	2.87	3.23	0.138	NS
(g/kg BW ¹)	12.60	12.29	11.73	12.93	0.573	NS
Concentrate DMI						
(kg/d)	1.35	1.33	1.27	1.28	0.060	NS
(g/kg BW)	5.28	5.26	5.18	5.10	0.204	NS
Digestibility (g/g)						
DM ¹	0.666 ^a	0.700 ^b	0.691 ^b	0.732 ^c	0.1183	***
OM ¹	0.682 ^a	0.715 ^b	0.707 ^b	0.748 ^c	0.0124	***
CP ¹	0.657	0.644	0.629	0.630	0.0164	NS
Ash	0.482 ^a	0.546 ^b	0.519 ^{a,b}	0.548 ^b	0.0253	*
Oil	0.643 ^{a,c}	0.606 ^a	0.711 ^{b,c}	0.631 ^{a,b}	0.0371	P=0.06
NDF ¹	0.647 ^a	0.692 ^b	0.677 ^b	0.707 ^b	0.0166	*
Seeds	0.918	0.934	-	-	0.0265	NS

¹ BW = bodyweight, DM = dry matter, OM = organic matter, CP = crude protein, NDF = neutral detergent fibre.

It is concluded that at the level of inclusion examined, the difference in oil digestibility when supplied as free oil or intact seeds would not warrant processing of seeds prior to feeding. With respect to CLA synthesis, the relative rates of oil release to the rumen merits investigation.

Moloney, A.P. and Monahan, F.J.¹

RMIS No. 5409

¹UCD School of Agriculture, Food Science and Veterinary Medicine, Belfield, Dublin

Long-term supplementation with sunflower/fish oil-containing concentrates in a grass-based beef production system: effects on colour and lipid stability during retail display

Increasing the concentrations of PUFA, including CLA, by nutritional strategies may adversely impact the colour and lipid stability of beef. Supplementary sunflower oil-containing concentrates have increased muscle CLA, while grazing and when combined with fish oil and wilted grass silage. To balance asynchronies between supply and demand and to maintain meat in acceptable condition, frozen storage is sometimes necessary, but this may also lead to less stable pigments and lipids. The objective was to determine the effect of (i) nutritional strategies designed to enhance CLA and (ii) frozen storage, on beef colour and lipid stability.

Sixty heifers were housed and fed a winter ration of either unwilted grass silage (U) with a standard barley/soyabean meal concentrate (SC) (USC) or wilted grass silage (W) with a concentrate containing sunflower oil (OC) (WOC). Following turnout to pasture heifers in both groups were offered one of three summer rations; either free access to pasture for 22 weeks (-PAST); a restricted grass (grazing) allowance with a sunflower oil-containing (20%) concentrate for 22 weeks (-PO22) or free access to pasture for the first 11 weeks and a restricted grass (grazing) allowance with a sunflower oil-containing (20%) concentrate for the final 11 weeks (-PO11). Samples of *M. longissimus dorsi* (LD) were recovered at 24 hours *post-mortem*. At 48 hours *post-mortem*, pH of LD was measured at the 7th rib and a section of LD (20cm) was removed between approximately the 5th and 8th ribs and bisected

longitudinally along the cranial caudal axis. A steak was recovered for chemical analysis. Both LD halves were aged for a further 19 days (21 days aging in total) when one half was placed in frozen storage (-20°C) for 8 months. Six steaks of 2.5cm were cut from the fresh half, packaged in a high O₂ atmosphere and placed in a refrigerated (<5°C), lighted (2800lm) retail display cabinet for 10 days. Hunter colour coordinates ('L' (lightness), 'a' (redness), 'b' (yellowness)) were measured at days 0 (approximately 3 hours after packaging), 1, 3, 6, 8 and 10. The 'C' (saturation) and 'H' (hue angle) values were calculated as $(a^2 + b^2)^{0.5}$ and $[\tan^{-1}(b/a)][180/\pi]$, respectively. Percent metmyoglobin and the difference between reflectance at 630nm and 580nm ($R_{630}-R_{580}$) were calculated as indices of discolouration. Lipid oxidation on days 0 and 10 was assessed as 2-thiobarbituric acid reactive substances (TBARS). The retail display trial was repeated after 8 months for the previously-frozen steaks. Data were analysed as a split-split-split plot design with main effects of winter ration and summer ration, freshness state (fresh or previously frozen) and days on display and all interactions included in the model.

Neither winter nor summer ration affected pH. LD from WOC heifers was more red (13.23 v. 12.62, SED = 0.197, P=0.003), yellow (8.48 v. 8.01, SED=0.207, P=0.027), saturated (15.82 v. 15.07, SED = 0.263, P=0.007), had higher $R_{630}-R_{580}$ (20.31 v. 18.74, SED=0.518, P=0.004) and lower metmyoglobin (29.06 v. 30.29, SED=0.525, P=0.024) than USC heifers. The effect of winter ration tended towards significance for lightness ('L' value; P=0.079). For all colour variables, there was a significant (P<0.001) interaction between freshness and day of display. There was no difference between initial redness, yellowness or saturation of fresh and frozen LD. For both fresh and frozen LD, redness, yellowness and saturation decreased (P<0.05) during display. Both fresh and frozen LD were less red (P<0.05) as the display period progressed although fresh LD was more red (P<0.05) than frozen LD on days 1, 3, 6, 8 and 10. There was no difference between redness on days 0 and 1 for fresh LD or between days 6 and 8 for frozen LD. For fresh LD, hue angle tended to increase until day 3 but increased significantly between days 6, 8 and 10 (all P<0.05) and hue angle of frozen LD was higher (P<0.05) than fresh LD at days 3, 6, 8 and 10. For frozen LD, hue angle increased (P<0.05) during display but was no different between day 3 for frozen LD and day 10 for fresh LD. There was a significant interaction between freshness and days on display for TBARS (P<0.001) whereby TBARS were higher on day 10 than on day 0 (P<0.05) but on day 10 the TBARS were higher (P<0.05) for the previously-frozen LD. LD from PO22 heifers had higher (P<0.05) TBARS at day 10 regardless of whether USC or WOC was fed over the winter.

It is concluded that feeding wilted grass silage with a sunflower oil-containing concentrate during winter increased redness, yellowness and saturation of LD. However, long term supplementation of grazing heifers with a sunflower oil-containing concentrate increased lipid oxidation. By all indices of colour stability, previously-frozen LD was less stable than fresh LD, regardless of diet.

Moloney, A.P., Dunne, P.G.¹ and Monahan, F.J.²

RMIS No. 5409

¹Teagasc, Ashtown Food Research Centre, Dublin

²UCD School of Agriculture, Food Science and Veterinary Medicine, Belfield, Dublin

Grange Beef Research Centre

Colour and lipid stability of beef from grazing or concentrate-fed cattle with and without supplemental alpha-tocopheryl acetate stored in a high oxygen atmosphere when intact or minced

Supranutritional supplementation of cattle with vitamin E has improved the colour and lipid stability of beef. Grass-fed cattle have muscle vitamin E concentrations which are 2 to 3 times greater than unsupplemented concentrate-fed cattle but also have higher concentrations of the highly oxidisable long chain n-3 PUFA. The objective of this study was to compare the oxidative stability of grass with concentrate-fed beef, both minced and intact, and to determine whether supplemental vitamin E can increase the oxidative stability of the beef during storage in a high oxygen modified atmosphere.

Forty continental crossbred heifers were blocked according to body weight (mean initial live weight 366 kg, SD = 35 kg) and within block, assigned randomly to one of four dietary treatments (n = 10 per treatment), i.e. two outdoor treatments – a restricted grazing allowance plus 2.5kg of supplementary concentrates containing either no added vitamin E (G0) or 400 I.U. (international units) of vitamin E, as alpha-tocopheryl acetate (G1000); two indoor treatments, receiving 70% of a basal concentrate ration plus 2.5kg of the same supplementary concentrates as the grazing heifers but with either no added vitamin E (C0) or 200 I.U. of vitamin E (C500). Following slaughter, samples of *M. longissimus dorsi* (LD) were excised from the 12th rib and a section was vacuum packaged and immediately frozen at -20°C. After 19 months of frozen storage, samples were thawed for 72 hours at 0°C. One half was cut into steaks and the other half was minced through a plate with 3mm holes and formed into patties. Steaks and patties were packaged in a high oxygen atmosphere (79.6%O₂:17.8%CO₂) and displayed for 10 days. Colour ('a' (redness), 'b' (yellowness), 'C' (saturation), 'H' (hue angle) and 'L' (lightness)) was measured on days 0, 1, 3, 6, 8 and 10 and lipid oxidation (as thiobarbituric acid reactive substances, TBARS) was measured on days 0, 3, 6 and 10. Vitamin E in LD muscle was measured. Data were analysed as a split-split-split plot with main effects of dietary treatment, comminution (intact or minced) and time (days) and all interactions included in the model. Where significant differences were detected means were compared using Fisher's least significance difference test.

Vitamin E concentrations in LD muscle were 1.46, 2.32, 2.84 and 2.90µgg⁻¹ for C0, C500, G0 and G1000, respectively, with no significant difference between the latter 3 treatments. There was a significant comminution × time interaction for all colour variables (P<0.001 for 'L', 'a', 'C', 'H' and P=0.007 for 'b') and for TBARS (P<0.001). Minced LD was initially more red (P<0.05) on days 0, 1 and 3 but less red (P<0.05) on day 8 than intact LD (Figure 14). There was a treatment × time interaction for 'a', 'H' (both P<0.001), 'C' (P=0.029) and TBARS (P<0.001). The C0 LD was less red (P<0.05) on day 3 and tended to be less red thereafter. The G0 LD tended to be most red on all days and both G treatments were more red (P<0.05) on days 6, 8 and 10. Minced LD had higher TBARS on day 0 (P<0.05) and days 3, 6 and 10 (all P<0.001) than intact LD (Figure 14). The G1000 LD had the lowest (P<0.05) TBARS on day 3 and 10 while the G treatments tended to have lower TBARS on days 6 and 10 PUFA.

It is concluded that while increased vitamin E stabilised lipids (indicated by lower TBARS values), it is not required to stabilise colour of grass-fed beef and that mincing causes increased discolouration and lipid oxidation.

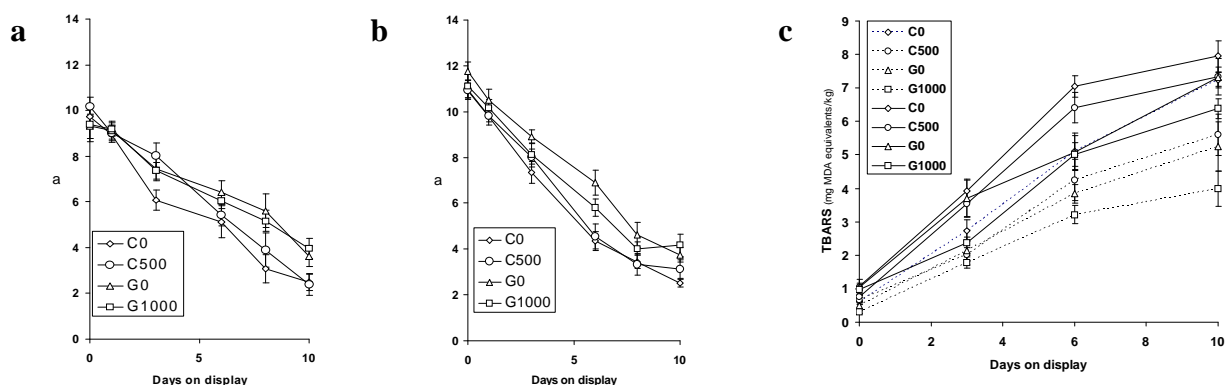


Figure 14. Change in redness of (a) intact and (b) minced LD muscle; (c) thiobarbituric acid reactive substances (TBARS) of intact (broken lines) and minced (solid lines)LD muscle.

Moloney, A.P., Dunne, P.G.¹ and Monahan, F.J.²

RMIS No. 5409

¹Teagasc, Ashtown Food Research Centre, Dublin

²UCD School of Agriculture, Food Science and Veterinary Medicine, Belfield, Dublin

Ovine intra- and inter-muscular variation in carbon turnover as recorded by stable isotope ratio analysis

Stable isotope ratio (SIR) analysis (SIRA) is used increasingly as a tool in food authenticity and food traceability. However, correct interpretation of observational data gathered in surveys requires a mechanistic understanding of underlying processes. This requires for example, controlled diet switching experiments, to establish the half-lives of the muscles used as meats.

In a controlled on-farm experiment, 28 lambs were switched from a C₃/C₄-control diet to a C₄-experimental diet (isotopic spacing between diets was 10.2 ‰ for C). Fourteen animals (7 males and 7 females) received a high-energy allowance (HEA), the other 14 (7 males and 7 females) animals received a low-energy allowance (LEA). Over the 33-week course of the experiment, animals were slaughtered at regular intervals to monitor the change in stable isotope ratios. Seven muscles (*Biceps femoris* (BF), *Cleidooccipitalis* (CO), *Flexor digitorum superficialis* (FDS), *Longissimus dorsi* (LD), *Psoas major* (PM), *Semimembranosus* and *Semitendinosus*) were collected 24 h *post mortem*, trimmed of superficial fat, weighed and vacuum packed. Statistical analysis of the muscle weights revealed that the energy allowance (EA) had a significant impact on all muscle weights ($p < 0.05$, $F > 6.45$), whereas the sex of the animal was significant for four muscles ($p < 0.01$, $F > 10.82$) and the duration of the experiment affected only three muscles significantly ($p < 0.05$, $F > 3.76$).

To determine inter-muscular variation, the most central location of five muscles (BF, CO, FDS, LD and PM) was sampled and C SIR measured. There was a significant difference in half-lives for the HEA and LEA ($p < 0.0001$, $t = -22.36$), with animals on the HEA having shorter half-lives than the animals on the LEA. However, not all five muscles showed the same half-lives. The half-life of the muscle FDS was shorter by more than 10% on the HEA and by more than 20% on the LEA compared to the other four muscles. To investigate any

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possible intra-muscular variation in $\delta^{13}\text{C}$, LD was sampled at ten different locations. Analysis revealed a significant difference between sampling locations along the muscle.

In conclusion, this experiment with lambs showed for the first time that the sampling location within one muscle has a significant impact on the results of a survey. We also showed that muscles typically consumed as human food have similar half-lives.

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Moloney, A.P., Harrison, S.M.^{1,2}, Monahan, F.J.¹, Kelly, S.D.³, Cuffe, F.⁴, Rossmann A.⁵, Schellenberg, A.⁶, Camin, F.⁷ and Schmidt, O.²

¹UCD School of Agriculture, Food Science and Veterinary Medicine, Belfield, Dublin

²UCD School of Biology and Environmental Science, Belfield, Dublin

³UEA School of Environmental Sciences, Norwich, NR4 7TJ, United Kingdom.

⁴UCD School of Mathematical Sciences, Belfield, Dublin

⁵Isolab GmbH, Schweitenkirchen, Germany

⁶LGL Bayern, Dienststelle Oberschleißheim, Erlangen, Germany

⁷IASMA Research Centre, Agrifood Quality Department, San Michele all'Adige, Italy

Comparison of subcutaneous fat colour and the colour and eating quality of sirloin steaks derived from beef animals reared on grass or concentrate diets

A study was undertaken to compare the colour of subcutaneous fat and the colour and eating quality of *longissimus dorsi* muscle (LD) from heifers reared on grass or concentrate diets. Fifty weaned, spring-born Charolais \times Limousin heifers were sourced in October/November 2006. The initial mean (SD) live weight (LW) was 275 (27.0) kg and the mean age was 252 (28.0) days. Heifers were blocked on initial LW and assigned at random, within block to either pasture or a concentrate-based diet. The experiment commenced at the beginning of December, 2006. For the pasture treatment, heifers rotationally grazed on herbage accumulated since the previous autumn (mainly *Lolium perenne*, *Poa* spp. and *Trifolium repens*) and offered fresh pasture daily. A dry matter (DM) allowance of ~3.5 kg DM/100 kg LW/d and a post-grazing pasture mass of 800-900 kg DM/ha were achieved by varying the size of the area grazed each day. From mid-April, the heifers at pasture were stocked in the same group. For the concentrate treatment, heifers were housed in a slatted-floor shed in groups of 5 and offered a restricted amount of concentrates (composition g/kg, rolled barley 430, molassed beet pulp 430, soyabean meal 80, molasses 35, minerals/vitamins 25) and 20% barley straw daily. All cattle were weighed at 4 week intervals. The strategy employed was for the pasture-fed cattle to achieve maximum potential growth and to restrict the allowance of concentrates and straw to the indoor group, such that both groups had a similar growth rate and ultimately carcass weight.

Cattle were slaughtered in October/November 2007. At 24 h *post-mortem*, carcass sides were cut at the 10th rib interface. A section (150 mm) of LD was recovered from the hind-quarter of each animal, vacuum packaged, transferred to Ashtown Food Research Centre and stored at 2°C for 14 days prior to frozen storage. An additional section of LD was recovered, vacuum packaged and transferred to Ashtown Food Research Centre for quality assessment. At 48 h *post-mortem*, pH was recorded in the centre of the muscle. A sample of LD (25 mm) was trimmed of adhering adipose tissue, overwrapped with oxygen-permeable PVC film and permitted to bloom at 4°C, in darkness for 3 h. Hunter Colour co-ordinates of 'L' (lightness), 'a' (redness) and 'b' (yellowness) values were taken on both adipose tissue and the bloomed muscles.

Vacuum packed frozen sections of LD (n = 23/diet, according to block) were forwarded to the Division of Farm Animal Science, University of Bristol. Upon arrival, samples were stored at -20°C until required for sensory assessment. The day before sensory assessment, LD were thawed in a refrigerator set at 1°C. On the morning of sensory assessment, LD were removed from their packs and steaks 1.9 cm thick were cut. Steaks, turning every 3 minutes, were cooked under a conventional grill until the internal temperature of the muscle reached 74°C

as measured by a thermocouple probe. Samples, approximately 2cm x 2cm x 1.9cm were then cut from the approximate centre of the steaks avoiding incursions of connective tissue where present. Samples were then wrapped in pre-coded foils and served hot to the 10 member Langford trained sensory panel. Assessors rated a descriptive flavour profile and also rated texture, juiciness, beef flavour intensity and abnormal flavour intensity using eight point category scales. Assessments took place in a purpose built panel room illuminated by red-light as a means of reducing bias that can occur if the cooked samples were viewed under normal light. Each booth contained a computer screen and optical mouse as part of the computerised sensory system, (Fizz, Version 2.10, Biosystemes, France), for direct entry of sensory responses. At each panel, assessors tasted 2 samples of LD steaks, half the assessors received a steak from a grass fed animal followed by a steak from a concentrate fed animal and *vice versa*. A total of twenty three panels were convened over four sessions.

Data were subjected to analysis of variance. For pH and colour co-ordinates, the model used had block and treatment as main effects. Based on muscle pH, two samples from the concentrate-fed group were deemed to be “dark-cutters” i.e. from animals that had experienced pre-slaughter stress. The data for muscle pH and colour were also analysed with these values excluded. For sensory data (n=23/treatment with dark cutters and samples from the corresponding blocks of grass-fed animals excluded), the model had treatment and assessor as main effects.

The mean duration of the study was 332 days. The mean carcass weight for both treatments was similar, as planned (Table 45). Carcasses from concentrate-fed heifers had better ($P<0.05$) conformation than those from grass-fed heifers (76%R, 4%O and 20%U vs 79%R and 21%O). Adipose tissue from grass-fed heifers was more yellow (higher ‘b’ value), more red (higher ‘a’ value), duller (lower ‘L’ value), had greater hue (higher ‘H’ value) and more intense colour (higher ‘C’ value) than adipose tissue from concentrate-fed cattle (Table 45).

Ultimate pH and colour co-ordinates of LD are shown in Table 46. There was no effect of pre-slaughter diet on ultimate pH. Muscle from grass-fed cattle was darker (lower ‘L’ value), less red (lower ‘a’ value), less yellow (lower ‘b’ value), had greater hue (higher ‘H’ value) and more intense colour (lower ‘C’ value) than muscle from concentrate-fed heifers.

The results from the sensory analysis are shown in Table 47. Steaks from concentrate-fed animals were more tender ($P<0.05$) than those from grass fed animals and differed by 0.3 units on an 8 point scale. Steaks from both treatments were in the ‘slightly tough to slightly tender’ categories. There was no significant differences between the two treatments in terms of juiciness, beef flavour intensity or abnormal flavour intensity. The steaks were assessed as ‘slightly to moderately juicy’ with ‘slightly weak to slightly strong’ beef flavour intensity. Abnormal flavour intensity was in the categories ‘moderately weak to very weak’. There were no differences attributed to flavour attributes between the grass and concentrate animals. In terms of overall liking there was a significant difference between the grass and concentrate fed animals whereby steaks from concentrate animals were preferred ($P<0.05$).

The ‘b’ value, or yellowness, can be considered the most important variable of fat colour. In this regard the findings of this study were consistent with those of previous studies in that the subcutaneous fat from grass – fed cattle was more yellow than that from concentrate – fed cattle. In contrast to previous studies at Grange Beef Research Centre, muscle from grazing cattle was darker and more red. Since muscle pH was similar, this difference does not reflect differences in pre-slaughter stress. Rather, it more likely reflects the duration of grazing (11 months) with a possible contribution of the physical activity engaged in by the grazing cattle, particularly during the summer when they were “set-stocked”.

It is concluded that that the absolute difference in ‘L’ and ‘a’ values is quite small and may not be visually detected by consumers. A significant difference was only observed in texture

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where steaks from concentrate-fed animals were more tender and this effect on tenderness was reflected in the preference ratings.

Table 45: Carcass characteristics of heifers offered grass or concentrate diets

	<u>Grass</u>	<u>Concentrates</u>	<u>S.e.d.</u>	<u>Significance</u>
Initial weight (kg)	275.5	273.0	2.49	NS
Final weight (kg)	512.7	506.1	7.63	NS
Carcass weight (kg)	270.2	276.8	4.23	NS
Conformation ¹	2.79	3.16	0.115	**
Fatness ¹	3.16	2.90	0.170	NS
'L'	67.60	71.40	0.573	***
'a'	11.07	8.61	0.410	***
'b'	24.99	17.65	0.488	***
'H'	66.25	64.17	0.750	*
'C'	27.36	19.66	0.557	***

¹Conformation: E = 5, P = 1; Fatness 1 = lean, 5 = fat (4L = 3.75).

²Higher values indicate lighter ('L'), more red ('a'), more yellow ('b'), greater hue ('H') and more intense/saturated ('C').

Table 46: Ultimate pH and colour¹ of *longissimus* muscle from heifers offered grass or concentrate diets²

	<u>Grass</u>	<u>Concentrates</u>	<u>s.e.d.</u>	<u>Significance</u>
pH ₄₈	5.52 (5.52)	5.56 (5.47)	0.070 (0.034)	NS (NS)
L	34.01 (34.25)	35.59 (35.99)	0.726 (0.725)	* (*)
a	17.16 (17.22)	17.95 (18.16)	0.408 (0.391)	0.064 (*)
b	14.47 (14.53)	15.56 (15.77)	0.377 (0.355)	** (**)
H	40.02 (40.03)	40.86 (40.92)	0.402 (0.408)	* (*)
C	22.46 (22.55)	23.77 (24.09)	0.531 (0.501)	* (**)

¹Higher values indicate lighter ('L'), more red ('a'), more yellow ('b'), greater hue ('H') and more intense/saturated ('C').

²Means in parentheses have samples with high pH values excluded.

Table 47: Sensory characteristics of grilled loin steak from heifers offered grass or concentrate diets

	<u>Grass</u>	<u>Concentrates</u>	<u>vr</u>	<u>Probability</u>	<u>Sig</u>
Attributes					
8 point scale used					
Texture	4.23	4.48	4.03	0.0454	*
Juiciness	5.23	5.31	0.66	0.4153	NS
Beef	4.51	4.41	0.93	0.3346	NS
Abnormal	3.24	3.10	1.14	0.2864	NS
100 mm line scales used					
Greasy	17.80	17.50	0.06	0.8055	NS
Bloody	10.60	9.70	0.68	0.4103	NS
Livery	14.86	15.28	0.09	0.7597	NS
Metallic	13.21	11.70	1.22	0.2692	NS
Bitter	9.65	8.66	0.67	0.4125	NS
Sweet	10.22	10.71	0.27	0.6030	NS
Rancid	4.52	6.01	2.16	0.1419	NS
Fishy	6.02	5.37	0.86	0.3547	NS
Acidic	12.90	11.90	0.53	0.4667	NS
Cardboard	17.08	16.17	0.52	0.4718	NS
Vegetable/Grass	17.11	16.75	0.08	0.7798	NS
Dairy	16.87	17.53	0.32	0.5730	NS
Hedonic					
Overall liking	38.95	42.28	4.15	0.0423	*

Values are the means derived from analysis of variance with Diet and assessor as factors with 23 replications * significant at 5%, **significant at 1%, *** significant at 0.1%

! least significance test post hoc not computed.

Moloney, A.P., Black, A., Dunne, P.G.¹ and Monahan, F.J.²

RMIS No. 5644

¹Teagasc, Ashtown Food Research Centre, Ashtown, Dublin

²UCD School of Agriculture, Food Science and Veterinary Medicine, Belfield, Dublin

Vitamin E levels in beef from different production systems and countries

The effectiveness of muscle vitamin E in delaying deteriorative changes, notably lipid and myoglobin oxidation, in meat is well established. In addition, muscle vitamin E levels are known to reflect the vitamin E content of the animals' diet pre-slaughter. More recently, interest in the potential for vitamin E to act as a marker for particular meat production systems has emerged and studies in beef cattle have shown that that the muscle vitamin E content of pasture-fed animals can be significantly higher than that of animals fed cereal-based diets. The objective of this study was to measure the vitamin E levels in muscle of beef cattle raised on feed components typically used in Ireland and to compare the levels obtained with those of beef from other countries.

Irish beef (*longissimus dorsi*) samples were taken from 98 beef cattle raised at Grange Beef Research Centre for 11 months pre slaughter either entirely on grass at pasture (n=24), entirely on a concentrate ration (n=25), silage (5 months) followed by grass (7 months) (n=24) or silage (5 months) followed by grass/concentrates (7 months) (n=25). Personal contacts and commercial suppliers were used to source non-Irish beef from Austria (n=6), Brazil (n=18), France (n=3), Germany (n=8) and the U.S. (n=12). Brazilian beef samples were obtained in 3 batches (I) and (II) in January 2007 and (III) in July 2007, with 6 samples

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analysed from each batch. Feed and muscle vitamin E (α -tocopherol) were measured by reverse phase HPLC. In muscle from Irish beef cattle raised on different diets, α tocopherol was higher in the muscle from grass (2.63 $\mu\text{g/g}$) and silage/grass (2.43 $\mu\text{g/g}$) fed animals compared to silage/grass/concentrate (1.77 $\mu\text{g/g}$) and concentrate (1.14 $\mu\text{g/g}$) fed animals. The higher levels of α -tocopherol in muscle from animals fed grass and grass/silage diets reflected a higher intake of α -tocopherol compared to the concentrate-fed animals. The mean α -tocopherol contents of the grass, silage and concentrate were 22.6, 63.4 and 36.6 mg/kg dry matter (DM), respectively, with 2-3 fold higher grass and silage DM intakes in the grass and silage-fed groups compared to concentrate DM intake in the concentrate-fed group. Mean muscle α -tocopherol was higher in the Brazilian samples (batches I to III) (8.13 mg/g) compared to European samples: Austrian (1.88 mg/g), French (2.64 mg/g), German (3.98 mg/g), the U.S. (2.26 mg/g) (Figure 15). The unexpectedly high α -tocopherol concentration in the Brazilian beef is currently being investigated.

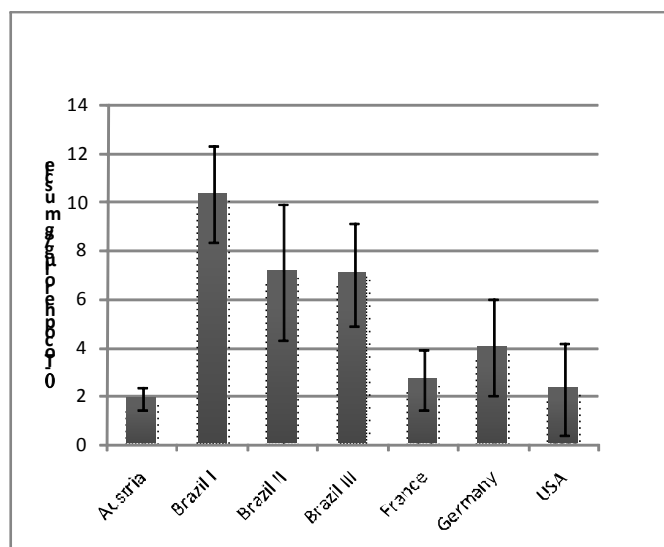


Figure 15. Vitamin E (α -tocopherol) of beef from different countries.

It is concluded that (i) the vitamin E content of beef from grass and grass/silage was higher than that of beef from animals fed concentrates (ii) there were similar vitamin E levels in Irish beef compared to other European beef and, (iii) there were 3 to 7 fold higher levels of vitamin E in Brazilian beef compared to European beef.

RMIS No. 5644

Moloney, A.P., Röhrle, F.T.¹, Lejeune, A.¹, Black, A., Sweeney, T.¹, Schmidt, O.² and Monahan, F.J.¹

¹UCD School of Agriculture, Food Science and Veterinary Medicine, Belfield, Dublin

²UCD School of Biology and Environmental Science, Belfield, Dublin

Influence of dietary vitamin E (α -tocopherol) on expression of genes involved in vitamin E and lipid metabolism in liver

Dietary vitamin E (α -tocopherol) is an important antioxidant in animal health, preventing oxidation of biomolecules such as DNA, lipids and proteins. Vitamin E also has non-antioxidant functions in transcriptional regulation. It could therefore regulate expression of genes involved in its own metabolism in liver (Figure 16). Previous work also suggests an involvement of vitamin E in stearoyl-CoA desaturase (SCD) gene expression, a key enzyme in the synthesis and regulation of CLA and PUFA. The objectives of this study were to determine (i) expression of genes coding for the alpha-tocopherol transfer protein (aTTP), cytochrome P450 3A4 (CYP3A4) and multidrug resistance protein 1 (MDR1) involved in

liver metabolism of vitamin E in cattle fed a concentrate based diet with or without vitamin E supplementation, (ii) the effect of vitamin E on the expression of the SCD gene using an *in vitro* human HepG2 hepatocyte cell line model.

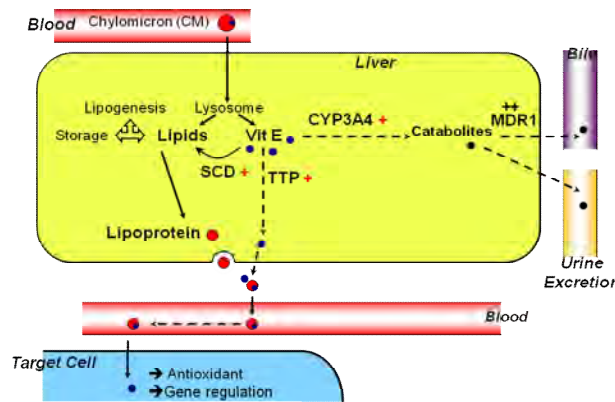


Figure 16. Vitamin E transport and metabolism in liver. TTP (Tocopherol Transfer Protein), CYP3A4 (Cytochrome P450 3A4), MDR1 (MultiDrug Resistance protein-1). This figure depicts previously suggested gene regulation by vitamin E (•) at the mRNA (+) or protein level (++).

Four groups of 8 continental heifers received diets with 0, 500, 1000, 3000 I.U. vitamin E supplementation/day for 160 days. RNA was extracted from liver and TTP, CYP3A4, MDR1 and SCD gene expression was measured using quantitative PCR. The effect of α -tocopherol (50 μ M, 500 μ M) was also tested on a human hepatocyte cell line (HepG2) over 24h. Arachidonic acid (200 μ M) was used as positive control of SCD1 and SCD5 gene regulation as it is known to inhibit SCD gene expression. RNA was extracted and gene expression measured using quantitative PCR.

Low vitamin E supplementation (500 I.U./day) tended to increase TTP gene expression ($P < 0.1$) and decrease MDR1 gene expression ($P < 0.1$) in comparison with the control (unsupplemented) group but no effect on these genes was observed with higher vitamin E supplementation (Figure 17). The high level of vitamin E (3000 I.U./day) tended to decrease SCD gene expression ($P < 0.1$). Expression of CYP3A4 was not affected by vitamin E supplementation. Figure 18 showed a trend toward a decrease of SCD gene expression upon treatment with arachidonic acid with the SCD1 gene being more down-regulated than SCD5. No clear evidence of a down-regulation in SCD1 or SCD5 gene in response to α -tocopherol at either 50 μ M or 500 μ M was observed.

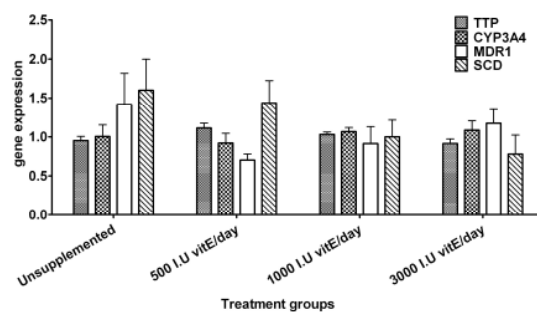


Figure 17. Effect of vitamin E supplementation on liver gene expression.

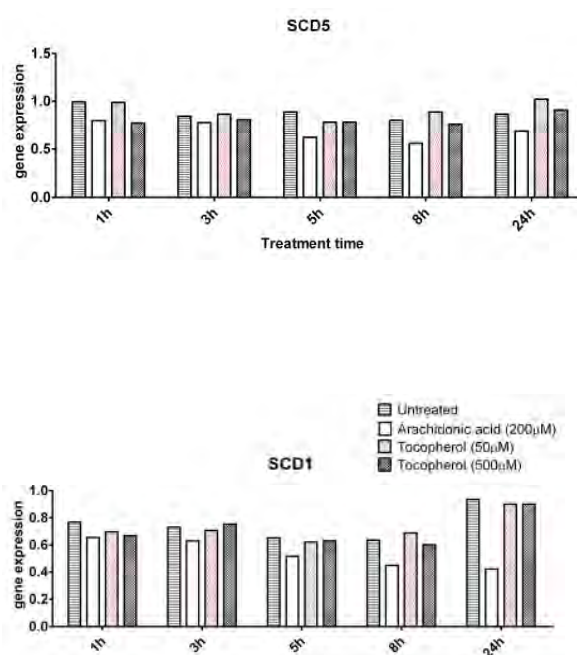


Figure 18. Regulation of human SCD1 and SCD5 in HepG2 cell by α -tocopherol.

This study did not reveal a strong association between vitamin E supplementation in cattle and the expression of genes involved in its own metabolism in liver. High levels of vitamin E supplementation could potentially decrease the expression of SCD, a key-enzyme in the synthesis and regulation of CLA and PUFA. As these lipids are highly beneficial for human health, further work should be carried out to assess their level in vitamin E-supplemented cattle. The preliminary study on a human hepatocyte cell line did not reveal clear evidence of modification of SCD1 and SCD5 genes following incubation with varying levels of α -tocopherol.

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Moloney, A.P., Lejeune, A.¹, McGettrick, S.¹, Sweeney, T.¹ and Monahan, F.J.¹

¹UCD School of Agriculture, Food Science and Veterinary Medicine, Belfield, Dublin

The inter-animal variation in ruminal lipid metabolism when cattle were offered a variety of rations

This study was undertaken to investigate the variation between animals in rumen microbial populations and the composition of fatty acids, in particular CLA, when animals were offered a range of contrasting rations. A pool of 15 Rotbunde x Friesian steers, fitted with ruminal cannulae were offered in sequence, a concentrate/straw ration (C), this ration supplemented with fish/plant oil (GO), grass (G) and grass supplemented with fish/plant oil (GO). During the final week of each period of 6 weeks duration, rumens were emptied on three consecutive days, (Day 1) 1 hour prior to feeding, (Day 2) 5 hours post-feeding and (Day 3) 11 hours post feeding. Both liquid and solid fractions were weighed and sampled to determine rumen pool sizes and samples of both fractions were taken at each evacuation for chemical analysis. During the evacuation on day 2 additional samples were collected for rumen bacterial analysis. Molecular analysis was carried out on bacterial samples from the solid and liquid fractions and on samples taken from the surface of the rumen epithelium using Terminal Restriction Fragment Length Polymorphism (TRFLP). Three restriction enzymes were used (Alu I, Hae III and Msp I) for TRFLP analysis.

Considering the poolsizes of individual VFA (Table 48), the variances were statistically similar across diets by sampling time except for the iso-valerate poolsize, the variances of which differed across diets at time 5 h (P<0.01). The ammonia poolsize had different variances across diets at time 5 h and 11 h but the variance of ammonia poolsize at time 0 h was not statistically different. For the lactic acid poolsize, the variances were significantly different across diets at time 5 h.

Table 48: Variances and mean values of fermentation end-product poolsizes for different diets before feeding 0, 5 and 11 hours after feeding

	Unit	Hours after feeding	C N=15		CO N=15		G N=14		GO N=14		Significance
			Variance	Mean	Variance	Mean	Variance	Mean	Variance	Mean	
L-Lactic acid poolsize	g	0	1.20	1.18	0.02	0.77	0.03	0.60	0.04	0.91	ns
		5	1.16	1.59	0.07	0.99	0.08	1.11	0.02	1.10	***
		11	0.03	0.71	0.02	0.67	0.04	0.66	0.06	0.74	ns
D-Lactic acid poolsize	g	0	1.48	1.58	0.03	0.97	0.09	0.60	0.03	0.84	ns
		5	1.35	1.92	0.18	1.40	0.16	1.06	0.06	1.02	***
		11	0.05	0.72	0.09	1.27	0.15	0.68	0.01	0.65	*
Lactic acid poolsize	g	0	5.29	2.77	0.07	1.73	0.15	1.19	0.11	1.76	ns
		5	4.91	3.50	0.43	2.39	0.38	2.17	0.09	2.12	***
		11	0.07	1.44	0.12	1.94	0.17	1.34	0.09	1.39	ns
Ammonia poolsize	g	0	9.86	5.88	2.47	4.74	0.87	2.64	1.83	1.90	ns
		5	7.88	4.17	2.57	1.90	9.31	5.38	2.51	2.13	*
		11	7.17	3.76	2.83	2.98	1.80	3.64	0.79	1.73	*
Acetate poolsize	mmol	0	248768.64	1909.97	137357.58	1728.94	250966.15	1686.51	677144.13	2919.73	ns
		5	391835.44	2202.74	260906.54	2580.24	765203.07	3571.09	372295.83	3207.99	ns
		11	449260.66	2540.68	263379.02	1972.88	262478.16	2633.50	184064.74	2413.45	ns
Propionate poolsize	mmol	0	11480.60	430.50	6176.78	424.59	10941.31	362.99	28627.34	666.30	ns
		5	48116.68	619.38	18908.14	765.94	55732.77	1035.73	29429.26	942.70	ns
		11	55028.59	647.21	14499.78	521.93	16262.43	597.78	11163.35	596.29	ns
Butyrate poolsize	mmol	0	14064.23	427.29	14124.53	321.17	9750.43	260.94	18000.76	467.69	ns
		5	36957.13	530.90	21519.70	499.97	43100.09	760.21	15736.72	699.68	ns
		11	35923.98	520.40	12240.02	368.28	13187.61	439.68	9489.47	432.39	ns
Iso-butyrate poolsize	mmol	0	602.04	57.79	660.97	60.39	854.14	57.55	1475.47	98.18	ns
		5	352.03	54.98	341.56	64.21	989.31	89.19	655.08	108.73	ns
		11	547.34	60.57	453.15	56.09	668.62	78.50	266.84	92.53	ns
Valerate poolsize	mmol	0	155.66	27.64	156.99	28.91	140.92	22.55	542.91	36.27	ns
		5	247.66	37.74	171.88	46.23	614.45	73.12	359.53	55.47	ns
		11	148.35	37.87	204.95	33.34	294.02	40.53	125.33	34.04	ns
Iso-valerate poolsize	mmol	0	306.42	51.12	315.77	56.32	312.94	43.75	874.57	67.16	ns
		5	387.51	47.64	418.98	62.49	2241.50	98.92	401.93	78.17	**
		11	288.91	51.41	350.83	52.87	604.42	69.30	235.38	61.74	ns

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Table 49 presents the variances and mean values for concentrations and pool sizes of rumen digesta components across diets before feeding and at 5 and 11 hours after feeding. The pool size at time 0 represents the feed pool size added to the rumen pool size before feeding. For all the data in the Table, the variances across diets were not significantly different at any sampling time.

Preliminary results restriction the microbial analysis suggest that diet and fraction have an effect on clustering for each enzyme but there was no obvious clustering effect due to animal. Further analysis of pooled data from all enzymes is in progress and the data will be statistically analysed using Canoco for windows, version 4.5. The main factors affecting bacterial community structure will be investigated using Principal Component Analysis (PCA) and a Monte Carlo Permutation test will be carried out to test for the effect of diet, animal and animal – animal variation. Fatty acid analysis is in progress and data will be superimposed onto the generated PCA plots to investigate the effect of clusters on fatty acids of interest.

Quantitative Polymerase Chain Reaction (QPCR) was also carried out on the total eubacterial, and stearate producing bacteria, *C. proteoclasticum*. In the solid fraction, total eubacteria and *C. proteoclasticum* were generally higher in both grass diets compared to the concentrate diets but neither were significantly different. In the liquid fraction, total eubacteria was highest for the concentrate diet supplemented with oil ($p < 0.0001$). However the opposite was seen for the stearate producing group where the concentrate appeared to have the lowest levels of *C. proteoclasticum*, but was not significantly lower than the other three diets. In the epithelial fraction, total eubacteria were significantly lower with the grass compared to the other diets ($p = 0.0003$) and there were no significant differences between all diets in *C. proteoclasticum* for the epithelial fraction.

Table 49: Variances and mean values of rumen digesta characteristics for different diets at different times before and after feeding

	Unit	Hours after feeding	C N=15		CO N=15		G N=14		GO N=14		Significance
			Variance	Mean	Variance	Mean	Variance	Mean	Variance	Mean	
Rumen content	Kg	0	60.01	42.21	36.37	43.25	48.30	32.45	61.00	47.79	ns
		5	59.35	51.28	48.55	50.47	70.49	56.75	65.34	56.24	ns
		11	53.64	48.20	38.64	47.33	37.21	50.53	26.13	48.81	ns
Dry matter	%	0	6.23	11.91	3.72	10.81	2.09	9.71	2.60	10.19	ns
		5	4.90	14.63	1.38	13.36	1.25	10.40	1.57	11.24	ns
		11	2.29	11.54	2.81	11.86	3.62	9.11	2.95	10.12	ns
Ash	g/Kg DM	0	134.17	111.20	416.35	113.05	121.86	134.54	144.43	133.43	ns
		5	46.79	92.26	90.77	95.41	111.41	120.96	58.19	127.71	ns
		11	133.17	103.14	105.92	102.28	218.63	132.30	78.71	131.38	ns
Crude Protein	g/Kg DM	0	761.70	176.53	174.85	173.10	208.60	235.59	307.66	202.59	ns
		5	176.19	209.37	91.89	172.72	120.80	240.21	95.29	186.67	ns
		11	389.80	201.89	176.19	209.37	189.52	246.23	208.20	197.50	ns
NDF	g/Kg DM	0	1670.33	624.19	1225.53	581.25	1052.16	505.52	1807.17	538.23	ns
		5	649.04	552.86	783.45	525.50	602.39	464.63	1711.53	506.65	ns
		11	1930.27	589.50	1498.30	573.82	1100.05	517.92	1453.17	527.51	ns
Dry matter poolsize	Kg	0	1.75	9.33	1.50	9.04	1.26	7.98	1.26	9.08	ns
		5	1.58	7.44	0.84	6.72	0.91	5.88	0.63	6.27	ns
		11	1.18	5.56	1.03	5.61	0.83	4.57	0.70	4.91	ns
Organic matter poolsize	Kg	0	1.43	8.35	1.32	8.09	1.00	7.22	1.01	8.06	ns
		5	1.28	6.76	0.75	6.08	0.71	5.17	0.50	5.47	ns
		11	1.03	4.99	0.90	5.04	0.68	3.97	0.55	4.27	ns
NDF poolsize	Kg	0	0.69	4.60	0.46	4.09	0.32	3.70	0.42	4.02	ns
		5	0.51	4.11	0.28	3.53	0.19	2.73	0.17	3.17	ns
		11	0.51	3.29	0.37	3.22	0.26	2.37	0.26	2.60	ns
Crude Protein poolsize	Kg	0	0.06	1.42	0.05	1.30	0.08	1.63	0.05	1.54	ns
		5	0.06	1.55	0.03	1.16	0.06	1.41	0.03	1.17	ns
		11	0.04	1.11	0.05	1.17	0.06	1.12	0.04	0.98	ns
Non-dry matter poolsize	L	0	51.32	37.23	30.50	38.59	38.81	29.29	55.23	42.98	ns
		5	49.89	43.84	38.89	43.75	59.12	50.87	58.39	49.96	ns
		11	43.11	42.64	31.28	41.72	35.64	45.96	24.11	43.89	ns

In conclusion, the results on rumen digestion suggest that the extent of variation is large and not homogeneous for variables describing rumen fermentation.

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Moloney, A.P., Grace, S., Joos, C.¹, Waters, S.M. Scollan, N.D.² and Huws, S.²

¹Wageningen Institute of Animal Sciences, Wageningen, The Netherlands

²Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Gogerddan Campus, Aberystwyth, Wales

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