Supplementary Material

The effect of cereal type and α-tocopherol supplementation on selective quality and processabiliy parameters of milk from late lactation grazing dairy cows.

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Supplementary materials and methods

Cows, treatments and experimental design

The cows were blocked on days in milk (DIM) (185 ± 19 DIM) and balanced for parity (2.60±1.65 lactations), milk yield (29.11±7.06 kg/d), milk composition (fat % (4.12±0.45%), protein % (3.57±0.24%), and fat and protein kg (2.21±0.42 kg/d)) and SCC (53±54 '000 cells/ml). The α-TOC levels (1050 IU α-TOC/d and 3150 IU α-TOC/d) in this study were selected based on data from two studies where similar levels of 0, 1500 and 3000 IU/d (Charmley and Nicholson, 1993a) and 0, 700 and 3000 IU/d (St-Laurent et al., 1990) were offered and where benefits were observed for increased milk fat concentration, reduced oxidised flavour and increased milk α-TOC concentration. The actual levels of α-TOC tested in the concentrate feed were lower than those formulated at 259 IU α-TOC /kg, 246 IU α-TOC /kg and 977 IU α-TOC /kg, which amounted to 777 IU α-TOC /d, 738 IU α-TOC /d and 2931 IU α-TOC/d for B, O and O+T respectively.

Cows had a 14 d dietary acclimatisation period which was followed by measurements taken weekly over 49 d for milk production and milk composition. Cows were offered the supplementary concentrate twice daily through the milking parlour (Feedrite automatic system,

Dairymaster, Kerry, IE) at a rate of 2.65 kg dry matter (DM)/cow/d (3 kg fresh weight). Cows were grazed in a single group and were offered fresh allocations of pasture twice daily (17 kg DM/d, total). Cows were observed while consuming the concentrates and given the low level of concentrate offered (1.33 kg morning and evening), animals consumed all concentrates offered and no refusals were recorded. Concentrates were manufactured by Gain Feeds (Portlaosie, IE). The average pre-grazing herbage mass was 1646 kg DM per hectare (ha) (above 4 cm), with an average pre-grazing compressed sward height of 10 cm (above ground level). The average post grazing mass was 492 kg DM per ha with an average post grazing compressed sward height of 5 cm. On a weekly basis, grass samples were pooled for proximate analysis. Weekly concentrate samples were taken and pooled for the duration of the trial to determine DM and then ground for use in analysis of proximate analysis.

Data and sample collection

Pasture and feed collection

All cows grazed permanent pasture (perennial ryegrass *Lolium perenne*) in a strip grazing system with pre-grazing herbage mass measured daily and before cows entered a new paddock using a rising plate meter (diameter 355 mm and 3.2 kg/m2; Jenquip, Fielding, New Zealand) by walking in a W shape across the field. Grass quality was determined using the quadrat and shears method as described by Whelan et al. (2012). Table 1 shows the ingredient and chemical composition of the feedstuffs offered. On a weekly basis, grass and concentrate samples were taken and pooled and ground for use in analysis of proximate analysis.

Milk sample collection

Cows were milked twice daily at 0700 h and 1500 h. Milk output and milk sampling were facilitated using the Weighall milk metering and sampling system (Dairymaster, Causeway, Kerry, IE). Milk samples were taken once weekly from one successive morning and evening

milking from each individual cow and pooled on a per cow basis according to test day milk yield. The individual milk samples were used to determine milk pH. Milk samples from each treatment (n=12) were pooled into three subsamples (n=4) where the cows in the three subsamples were balanced for parity, milk kg and SCC. Pooled milk samples were analysed for fat, protein, casein, lactose, SCC, urea, milk fatty acid profile, RCT, ES, minerals and α-TOC.

Sample analysis

Pasture and feed sample analysis

Pasture and concentrate samples were dried in a forced air oven at 55 °C for 3 d and were ground in a hammer mill fitted with a 1 mm screen (Lab Mill; Christy Turner, Suffolk, UK). The DM content of samples was determined by drying at 105 °C overnight (16 h minimum) (AOAC International, 2005c, 930.15). Ash was determined following combustion in a muffle furnace (Nabertherm GMBH, Lilienthal, DE) at 550 °C for 5.5 h (AOAC International, 2005a, 942.05). Neutral detergent fibre and ADF were determined using the method of Van Soest et al. (1991) adopted for use in the Ankom™ 220 Fibre Analyser (Ankom™ Technology, NY, USA). Gross energy was determined by bomb calorimetry (Parr 1281 bomb calorimeter, Parr Instrument Company, Moline, Illinois, US). Ether extract was determined using Soxtex instruments (Tecator, Hoganas, SE) and light petroleum ether. The CP content of the pasture and concentrate samples was determined by combustion (FP 528 Analyzer, Leco Corp, St Joseph, Michigan, US; AOAC International, 2005b, 990.03). Starch content of feed samples was analysed using the Megazyme Total Starch Assay Procedure (product no: K-TSTA; Megazyme International Ireland LTD, Wicklow, IE). The α-TOC content of feed and pasture was analysed by an UKAS accredited method by ALS Global (Carrigeen Business Park, Suite 1, Clonmel, Co. Tipperary).

Milk sample analysis

Concentrations of milk fat, protein, lactose, casein, urea and SCC over the 49 d of the study were determined in a commercial milk laboratory (National Milk Laboratories Ltd, Unit 26-29, Laches Close, Calibre Industrial Park, Four Ashes, Wolverhampton, UK, WV10 7DZ) using mid-infrared spectrometry (MilkoScan FT6000, Foss Analytical A/S, Hillerod, DK; Soyeurt et al. 2006). Measurements were taken on weeks 3 and 7 for pooled milk fatty acid profile analysis which was determined in a commercial laboratory using a variation of the Bligh and Dyer (1959) method for total lipid extraction and purification (Agri-Food and Biosciences Institute, Newforge Lane, Belfast, BT9 5PX). Milk pH was analysed using the Phoenix Instrument EC-25 pH/ Conductivity Portable Meter and averaged per d. The dl a-tocopherol acetate content of milk samples was also analysed by ALS Global (Carrigeen Business Park, Suite 1, Clonmel, Co. Tipperary). Rennet coagulation time was determined by modification of the method by Berridge (1952). Briefly, five mL of rennet was diluted with 100 mL of distilled water to give a 1/20 rennet dilution (Naturen (R) Chr. Hansen, Little Island, Cork containing 1.45 IMCU/ml. For each milk sample, 5 mL was measured into a test tube and placed in a water bath to allow a 5 min equilibrium time to reach 30 °C. Once the samples had reached 30 °C 0.5 mL of the rennet dilution was added (0.752 IMCU/ml milk in the actual test) and the timer started simultaneously. The sample was slowly inverted twice, attached to a rotating holder and immersed in the water bath at a 30 ° angle with rotation set to max speed (4 rpm). The length of time taken for milk to coagulate was recorded. Ethanol stability (ES) was determined using the method reported by Guo et al. (1998). The dl a-tocopherol acetate content of feed and milk samples was analysed using an UKAS accredited method of extracting dl a-tocopherol acetate from the feed using ammonia/methanol solution at 60 °C, shaken with chloroform and filtered. The diluted extract is determined by reverse phase HPLC with fluorescence detection. The mineral profile of milk samples was also analysed by ALS Global (Carrigeen Business Park,

Suite 1, Clonmel, Co. Tipperary) using the UKAS accredited method adapted from the Thermo Fisher application.

Statistical analysis

Data was checked for adherence to the normal distribution and homogeneity of variance using histograms and formal statistical tests as part of the Univariate procedure of SAS (9.3 2012). The natural logarithm transformation of milk SCC was used to normalize the distribution. The transformed data were used to calculate P-values. However, the corresponding least squares means and standard errors of the non-transformed data are presented in results for clarity (Al Ibrahim et al., 2010). Analysis of data was conducted using Proc Mixed of SAS (2012). The model included the fixed effects of treatment and week and their interaction. The interaction of treatment*time was non-significant in the model and is therefore not reported in this paper.

Statistically significant differences between least squares means were tested using the PDIFF command incorporating the Tukey test for pairwise comparison of treatment means. The model was adjusted for multi-comparisons using Bonferroni. Repeated measures (day) and random effects (sub-sample) were also included in the model. Statistical significance was assumed at a value of $P < 0.05$ and a tendency toward significance assumed at a value of $P > 0.05$ but < 0.10.

Table S1: Ingredient and chemical composition of experimental feedstuffs

	Experimental Feedstuffs			
	Barley[1]	Oats [2]	α-Tocopherol [3]	Pasture
Ingredient Composition g/kg DM				
Barley	420	-	-	-
Oats	-	420	420	-
Maize distiller	117	117	117	-
Soyabean meal 47 %	115	115	115	-
Soya hulls	117	117	117	-
Palm kernel expeller	117	117	117	-
Palm oil	4	6	6	-
Sugarcane molasses	50	50	50	-
Mono DCP	4	4	4	-
Calcium carbonate	14	14	-	-
Sodium chloride	7	7	7	-
Magnesium oxide	20	20	20	-
Vitamin E premix[4]	7	7	21	-
Gain cattle premix[5]	8	8	8	-
Chemical Composition g/kg DM				
DM	870	876	874	135
Crude protein	172	169	171	217
Ash	98	95	105	93
NDF[6]	314	349	361	499
ADF[7]	160	187	199	238
Ether extract	13	12	13	18
WSC[8]	-	-	-	139
Starch	211	181	170	-
Gross energy MJ/kg	17.1	17.4	17.3	17.4
α-Tocopherol IU/kg	259	246	977	44

[1] Barley based concentrate + 350 IU α-TOC/kg (B)
[2] Oat based concentrate + 350 IU α-TOC/kg (O)
[3] Oat based concentrate + 1050 IU α-TOC/kg based concentrate (O+T).
[4] DSM's Rovimix E adsorbate (dl-alpha tocopheryl acetate)
[5] Gain Cattle premix consistent of the following: **Barley:** 1.38g calcium, 0.51g phosphorus, 0.31g sodium, 1.02g potassium, 0.66g chlorine, 1.20g magnesium, 0.07g copper, 0.02g iodine 0.12g manganese, 0.001g selenium, 0.23g sulphur, 0.22g Zinc, 12,000IU vitamin A, 3000IU vitamin D; **Oats and α-tocopherol;**: 1.40g calcium, 0.51g phosphorus, 0.31g sodium, 0.99g potassium, 0.65g chlorine, 1.22g magnesium, 0.07g copper, 0.02g iodine 0.13g manganese, 0.001g selenium, 0.24g sulphur, 0.23g Zinc, 12,000IU vitamin A, 3000IU vitamin D.
[6] Neutral detergent fibre (NDF)
[7] Acid detergent fibre (ADF)
[8] Water soluble carbohydrates (WSC)

Table S2: The effect of treatment on milk composition and processability

	Treatment (n=4)					
	Control (C)[1]	Barley (B)[2]	Oats (O)[3]	α-tocopherol (O+T)[4]	SEM	P-Value
Processability						
Milk pH	6.62	6.61	6.60	6.62	0.008	0.07
Milk Composition (%)						
Fat	4.90	4.70	4.86	4.84	0.056	0.06
Protein	3.93	3.90	3.95	3.92	0.039	0.85
Casein	3.13	3.12	3.17	3.14	0.036	0.78
Casein as % total protein	79.5[a]	80.0[b]	80.3[b]	80.1[b]	0.267	0.01
Lactose	4.13[a]	4.19[b]	4.25[c]	4.28[c]	0.017	<0.001
Urea (mg/dl)	33	34	33	32	0.60	0.36
SCC (‘000 cells/ml)5	109	104	113	98	12.16	0.71
Mineral profile mg/100g						
α-tocopherol	0.19	0.20	0.20	0.20	0.007	0.75
Calcium	128	123	130	138	3.16	0.39
Magnesium	12.15	11.80	12.25	12.23	0.440	0.83
Potassium	149	149	152	148	2.460	0.71
Sodium	45.55	42.28	40.78	40.28	1.517	0.06
Zinc	0.374	0.371	0.417	0.388	0.015	0.07
Phosphorus	94.95	92.40	97.18	98.38	2.332	0.13

abc within a row, means with different superscripts differ ($P < 0.05$)

[1] Control, pasture only (C)

[2] Barley based concentrate + 350 IU α-TOC/kg (B)

[3] Oat based concentrate + 350 IU α-TOC/kg (O)

[4] Oat based concentrate + 1050 IU α-TOC/kg based concentrate (O+T)

[5] For SCC the natural logarithm transformation data were used to calculate P-values. The corresponding least squares means and standard errors of the non-transformed data are presented in results for clarity

Table S3: The effect of treatment on the full milk fatty acid profile

	Treatment (n=4)					
	Control (C)[1]	Barley (B)[2]	Oats (O)[3]	α-tocopherol (O+T)[4]	SEM	P-Value
% of total fatty acids						
SFA						
Butyric acid (C4:0)	2.07	2.15	2.11	2.14	0.033	0.27
Caproic acid (C6:0)	1.41[a,b]	1.46[a]	1.46[a]	1.38[b]	0.026	0.05
Caprylic acid (C8:0)	0.85[a]	0.91[a]	0.89[a]	0.79[b]	0.023	0.005
Capric acid (C10:0)	2.03[a]	2.18[a]	2.14[a]	1.80[b]	0.071	0.005
Undecanoic acid (C11:0)	0.04[a]	0.04[a]	0.04[a]	0.03[b]	0.003	0.01
Lauric acid (C12:0)	2.54[a]	2.93[b]	2.81[b]	2.40[a]	0.084	0.002
Tridecanoic acid (C13:0)	0.08[a]	0.07[a]	0.07[a]	0.06[b]	0.004	0.03
Myristic acid (C14:0)	9.86[a,c]	10.55[b]	10.14[a,b]	9.47[c]	0.187	0.006
Pentadecanoic acid (C15:0)	1.15[a]	1.15[a]	1.06[b]	1.09[b]	0.016	0.002
Palmitic acid (C16:0)	27.08	27.85	27.19	27.15	0.523	0.54
Heptadecanoic acid (C17:0)	0.70[a]	0.64[b]	0.64[b]	0.66[b]	0.014	0.0002
Stearic acid (C18:0)	11.69[a,b]	11.08[b]	12.20[a]	11.97[a]	0.267	0.02
Arachidic acid (C20:0)	0.138	0.140	0.143	0.140	0.003	0.77
Henicosanoic acid (C21:0)	0.058	0.057	0.055	0.057	0.001	0.45
Behenic acid (C22:0)	0.065	0.067	0.068	0.070	0.002	0.40
Tricosanoic acid (C23:0)	0.03	0.03	0.03	0.03	0.0008	0.42
Lignoceric acid (C24:0)	0.04	0.04	0.04	0.04	0.008	0.77
MUFA						
Myristoleic acid (C14:1)	1.08[a]	1.15[a]	0.96[b]	1.08[a]	0.027	0.002
Palmitoleic acid (C16:1)	2.28	2.29	2.21	2.19	0.058	0.51
Heptadecenoic acid,C17:1c10	0.008[a]	0.002[b]	0.007[a]	0.008[a]	0.002	0.04
Oleic acid (C18:1 cis 9)	27.19	25.05	26.32	26.83	0.606	0.10
Octadecenoic acid (C18:1 cis 11)	0.54[a]	0.43[b]	0.50[a,c]	0.48[c]	0.019	0.0008
Elaidic acid (C18:1 trans 9)	0.20	0.21	0.21	0.20	0.006	0.34
Vaccenic acid (C18:1 trans 11)	4.16[a]	4.55[a,b]	4.09[a]	4.96[b]	0.250	0.05
Paullinic acid (C20:1 c11)	0.047[a,b]	0.042[b]	0.050[a]	0.048[a]	0.002	0.03
Erucic acid (C22:1 c13)	0.022[a]	0.035[b]	0.030[b,c]	0.027[a,c]	0.003	0.02
Nervonic acid (C24:1c15)	0.015	0.018	0.012	0.013	0.002	0.16
PUFA						
Linolelaidic acid (C18:2 trans)	0.02	0.02	0.02	0.02	0.001	0.53
Linoleic acid (C18:2 c9 t12)	1.20	1.30	1.27	1.27	0.043	0.35
γ-linolenic acid (C18:3 c6, 9, 12)	0.02	0.02	0.02	0.02	0.001	0.30
α-linolenic acid (C18:3 9,12,15)	0.92	0.82	0.86	0.81	0.041	0.15
Eicosadienoic acid (C20:2 c11 c14)	0.02	0.02	0.02	0.02	0.0008	0.42
Dihomo-γ-linolenic acid (C20:3c8,11, 14)	0.052[a]	0.065[b]	0.058[c]	0.057[c]	0.001	<0.001
Eicosatrienoic acid (C20:3 c11,14,17)	0.02	0.02	0.02	0.02	0.002	0.21
Arachidonic acid(C20:4 c5,8,11,14)	0.08[a]	0.09[b]	0.08[a]	0.08[a]	0.002	0.003

Eicosapentaenoic acid (C20:5 c5,8,11,14,17)	0.082	0.078	0.077	0.077	0.004	0.68
Docosapentaenoic acid (C22:5 cn3 7, 10, 13, 16,19)	0.113	0.118	0.112	0.108	0.006	0.57
Docosahexaenoic acid (C22:6 c4,7,10,13,16,19)	0.012	0.007	0.010	0.010	0.002	0.09
Conjugated Linoleic acid (CLA)						
CLA (c9, t11)	2.09[a,b]	2.40[a,c]	1.98[b]	2.41[c]	0.154	0.03
CLA (t10, c12)	0.01	0.01	0.01	0.01	0.001	0.69
Total SFA	59.86	61.07	59.67	60.86	0.751	0.25
Total MUFA	31.51[a]	29.75[b]	30.68[a,b]	28.99[b]	0.301	0.04
Total PUFA	2.52	2.52	2.55	2.46	0.06	0.72
Total CLA	1.89[a]	2.20[a,b]	2.21[b]	2.62[c]	0.153	0.003
Omega 3 (N3)	1.06	1.16	1.01	1.06	0.04	0.07
Omega 6 (N6)	1.46[a,b]	1.36[a]	1.54[b]	1.40[a]	0.04	0.03
Omega 7 (N7)	2.84	2.70	2.70	2.69	0.07	0.34
Omega 9 (N9)	27.76[a]	25.93[b,c]	26.96[a,b]	25.10[c]	0.608	0.03

abc within a row, means with different superscripts differ ($P < 0.05$)

[1] Control, pasture only (C)

[2] Barley based concentrate + 350 IU α-TOC/kg (B)

[3] Oat based concentrate + 350 IU α-TOC/kg (O)

[4] Oat based concentrate + 1050 IU α-TOC/kg based concentrate (O+T)