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Effect of Feeding Intensity and Milking System on Nutritionally Relevant Milk Components in Dairy Farming Systems in the North East of England

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ABSTRACT: There is increasing concern that the intensification of dairy production reduces the concentrations of nutritionally desirable compounds in milk. This study therefore compared important quality parameters (protein and fatty acid profiles; α-tocopherol and carotenoid concentrations) in milk from four dairy systems with contrasting production intensities (in terms of feeding regimens and milking systems). The concentrations of several nutritionally desirable compounds (β-lactoglobulin, omega-3 fatty acids, omega-3/omega-6 ratio, conjugated linoleic acid c9t11, and/or carotenoids) decreased with increasing feeding intensity (organic outdoor $\geq$ conventional outdoor $\geq$ conventional indoors). Milking system intensification (use of robotic milking parlors) had a more limited effect on milk composition, but increased mastitis incidence. Multivariate analyses indicated that differences in milk quality were mainly linked to contrasting feeding regimens and that milking system and breed choice also contributed to differences in milk composition between production systems.

KEYWORDS: *dairy management, robotic milking, milk protein, fatty acid profile, antioxidants, carotenoids*

INTRODUCTION

Milk and dairy products are important components of the human diet, providing a wide range of nutrients (protein, fat, and carbohydrates) and nutritionally desirable functional compounds including bioactive peptides, vitamins, antioxidants, minerals, phytosterols, conjugated C18:2 c9t11 (rumenic acid; RA), and omega-3 (n-3) fatty acids (FA).[1,2] The protein composition of milk is ideal for the needs of mammalian neonates,[3] and milk proteins and bioactive peptides, originating from milk casein and whey protein digestion, are associated with antimicrobial, antiviral, opioid, anticarcinogenic, antihypertensive, antithrombotic, and immunomodulating actions.[4] However, certain saturated fatty acids (SFA) in milk, such as C12:0 (lauric acid, c12), C14:0 (myristic acid, c14), and C16:0 (palmitic acid, c16), have been linked to an increased risk of cardiovascular diseases (CVD) and metabolic syndrome.[1,3,5] In contrast, certain monounsaturated FA (MUFA) and polyunsaturated FA (PUFA) have been linked to a reduced risk of CVD, certain cancers, obesity, and type 2 diabetes and enhanced immune system function.[1,5−8] Milk fat also contains antioxidants/vitamins (e.g., carotenoids and α-tocopherol). Although these are linked to protection against oxidative stress, CVD, certain cancers, cataracts, and other health benefits, it is important to point out that milk/dairy products are considered to be a less important dietary source of these compounds than fruits and vegetables.[1,9]

A range of studies have investigated the effect of feeding regimens and milking frequency on milk fat composition. For example, high concentrate and conserved forage-based diets and increased milking frequency were shown to reduce concentrations of PUFA, n-3, and RA and vitamin/antioxidant concentrations in milk.[5,7,10,11] Changes in dairy diets were also shown to affect milk protein concentration and composition,[12,13] and increased milking frequency was shown to decrease concentrations of both PUFA and total milk protein.[14] However, there are to our knowledge no studies into the effects of contrasting dairy production systems on protein composition.

Most studies into the effect of dairy farming systems have focused on comparing milk from organic and conventional farming systems and reported higher concentrations of nutritionally desirable n-3, with some studies also reporting higher concentrations of RA, α-tocopherol, and/or carotenoids, in organic milk.[5,7,10] However, in a recent cross-European study

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Table 1. Management and Production Parameters for the Three Dairy Systems Using Standard Milking Parlors but Contrasting Feeding Intensities (OOS, Organic Outdoors; COS, Conventional Outdoors; CIS, Conventional Indoors) in Two Seasons (Indoor, December, February, March; Grazing, May, June, July) in the North East of England (Mean Values ± SE)

	feeding intensity (F)			season (S)		ANOVA P values[a]		
parameter assessed	OOS (n = 29)	COS (n = 28)	CIS (n = 30)	indoor (n = 58)	grazing (n = 59)	F	S	F × S
herd size (no. of cows)	105 ± 8 b	139 ± 12 b	341 ± 21 a	199 ± 21	196 ± 19	**	ns	†
yield (kg/cow/day)	23.4 ± 1.2	27.6 ± 0.6	29.5 ± 0.5	26.6 ± 0.9	27.2 ± 0.7	†	ns	ns
milk composition								
fat (g/kg milk)	39.9 ± 0.6	39.7 ± 0.4	38.9 ± 0.5	40.4 ± 0.3	38.6 ± 0.4	ns	***	ns
protein (g/kg milk)	33.1 ± 0.2 a	33.4 ± 0.3 a	32.1 ± 0.2 b	32.6 ± 0.2	33.1 ± 0.2	*	*	ns
SCC (×10^3/mL milk)	207 ± 13	197 ± 9	177 ± 9	192 ± 10	195 ± 8	ns	ns	ns
urea (g/kg milk)	0.21 ± 0.01	0.25 ± 0.02	0.29 ± 0.01	0.26 ± 0.01	0.25 ± 0.01	ns	ns	ns
nutrition (% of DMI)								
estimated grazing	36.6 ± 7.1 a	19.7 ± 4.3 b	3.1 ± 1.7 c	0.4 ± 0.4	38.4 ± 4.8	***	***	***
conserved forage[b]	40.9 ± 5.8	41.7 ± 3.2	50.1 ± 1.6	59.7 ± 1.6	29.3 ± 2.7	**	***	***
grass silage	35.5 ± 4.9	35.7 ± 3.3	31.2 ± 1.4	47.2 ± 2.1	21.3 ± 1.9	ns	***	***
maize silage	0.2 ± 0.2	0.0 ± 0.0	9.0 ± 2.2	4.0 ± 1.4	2.4 ± 1.0	†	*	*
cereal whole crop[c]	4.1 ± 1.3	2.7 ± 1.0	8.8 ± 0.9	6.4 ± 1.0	4.2 ± 0.9	†	**	***
straw/hay	1.1 ± 0.3 b	3.4 ± 0.4 a	1.1 ± 0.3 b	2.2 ± 0.3	1.5 ± 0.4	*	*	†
concentrates	22.5 ± 1.9 b	39.6 ± 1.8 a	46.8 ± 1.6 a	39.9 ± 1.7	33.0 ± 2.4	***	***	***
supplements (kg/cow/day)								
mineral/vitamins	0.05 ± 0.01 b	0.26 ± 0.05 a	0.15 ± 0.02 ab	0.17 ± 0.03	0.13 ± 0.02	*	ns	ns
lipid[d]	0.00 ± 0.00	0.15 ± 0.04	0.22 ± 0.04	0.11 ± 0.02	0.14 ± 0.03	ns	ns	ns
health parameters (% of total cows)								
mastitis incidences	4.8 ± 0.8	1.6 ± 0.4	2.5 ± 0.6	3.2 ± 0.5	2.8 ± 0.6	ns	ns	ns
mastitis treatments	3.1 ± 0.5	1.6 ± 0.4	2.4 ± 0.6	2.4 ± 0.4	2.3 ± 0.5	ns	ns	ns
other health treatments	6.2 ± 2.1	1.5 ± 0.4	2.1 ± 0.7	3.4 ± 0.9	3.2 ± 1.2	ns	ns	ns

[a]Significances were declared at ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; and †, $0.05 < P < 0.10$ (trend); ns, $P > 0.10$ (nonsignificant). Means for systems with different feeding intensities within rows and with different letters are significantly different ($P < 0.05$) according to Tukey's honestly significant difference test. [b]Tukey's honestly significant difference test did not show significant differences between the means of different feeding intensity systems, although the impact of feeding intensity on conserved forage intake was declared significant by ANOVA. [c]Ensiled whole wheat plants (stem, leaves, and immature grain), harvested approximately 1 month before grain maturity. [d]Megalac, a commercial product consisting of extracted palm oil (mainly palmitic acid) and calcium.

differences in FA composition between countries were reported to be greater than between organic and conventional systems within countries, and this was linked to differences in production intensity (e.g., the level of concentrate feed supplementation and conserved forage in the diet).[15] There have been few studies comparing milk composition in conventional systems with contrasting production intensity, and the studies available focused mainly on quantifying the effect of introducing more extensive grazing-based management systems, which was shown to increase the concentrations of nutritionally desirable n-3, RA, and/or fat-soluble antioxidants in milk.[7,11] In contrast, there have been to our knowledge no detailed studies into the effect on intensifying dairy production (e.g., year-round indoor production and associated diet changes and/or use of robotic milking systems) on nutritionally relevant compounds in milk (e.g., protein composition, FA profiles, and antioxidant concentrations). There is also limited information on the effect of breed choice on milk composition/quality,[16−18] although the potential to improve milk composition via breeding/genetic selection was recently demonstrated.[18]

To overcome these gaps in knowledge the three main aims of the study were to (a) quantify the effects of feeding regimen intensification (by comparing three farming systems with increasing concentrate and decreasing grazing based dry matter intake (DMI) in the diet) on nutritionally relevant milk composition parameters (protein, FA, and antioxidant profiles); (b) quantify the effect of milking system (by comparing farms using standard and robotic milking systems but similar feeding regimens) on animal health and nutritionally relevant milk composition parameters (protein, FA, and antioxidant profiles); and (c) identify associations between specific production system components (e.g., dietary components, housing, milking system/frequency, proportion of Holstein-Friesian cows) and milk composition by redundancy analysis.

MATERIALS AND METHODS

Experimental Design. A farm survey approach based on the methodology developed by Butler et al.[7] was used. The survey was conducted in the North East of England and included farms representing the four main production systems used by dairy farms in the region: (a) organic outdoor with standard milking parlor (OOS), (b) conventional outdoor with standard milking parlor (COS), (c) conventional outdoor with robotic milking (COR), and (d) conventional indoor with standard milking parlor (CIS) (see below for detailed production system descriptions). Feeding intensity (concentrate/grazing ratio) was lowest in outdoor organic, intermediate in outdoor conventional, and highest in indoor conventional systems (see Table 1). The two outdoor conventional systems (COS and COR) had similar feeding regimens, but differed in milking systems (standard vs robotic milking, Table 2), resulting in contrasting milking frequency. A total of 20 farms, 5 farms per production system, were included in the survey. Milk yield and standard compositional parameters and details of dietary and husbandry/management regimens (including mastitis incidence, somatic cell count (SCC), and antibiotic treatments for mastitis and other health parameters) were recorded/collected (a) at three dates (approximately 8 weeks apart) during the outdoor grazing season (when cows in the three outdoor systems were grazed on pasture) and (b) at three dates during the indoor season (when cows from all four systems

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Table 2. Management and Production Parameters for Dairy Systems of Similar Feeding Intensities and Different Milking Systems (COS, Conventional Outdoors with Standard Milking Systems; COR, Conventional Outdoors with Robotic Milking Systems) in Two Seasons (Indoor, December, February, March; Grazing, May, June, July) in the North East of England (Mean Values ± SE)

	milking system (M)		season (S)		ANOVA P values[a]		
parameter assessed	COS (n = 28)	COR (n = 30)	indoor (n = 28)	grazing (n = 30)	M	S	M × S
herd size (no. of cows)	139 ± 12	82 ± 6	110 ± 11	109 ± 10	ns	ns	ns
yield (kg/cow/day)	27.6 ± 0.6	26.3 ± 0.6	26.6 ± 0.6	27.3 ± 0.7	ns	ns	ns
milk composition							
fat (g/kg milk)	39.7 ± 0.4	39.1 ± 0.4	40.7 ± 0.3	38.2 ± 0.3	ns	***	†
protein (g/kg milk)	33.4 ± 0.3	32.2 ± 0.3	32.3 ± 0.3	33.2 ± 0.3	*	**	ns
SCC ($\times 10^3$/mL milk)	197 ± 9	212 ± 13	205 ± 11	205 ± 13	ns	ns	ns
urea (g/kg milk)	0.25 ± 0.02	0.24 ± 0.01	0.25 ± 0.02	0.25 ± 0.01	ns	ns	ns
nutrition (% of DMI)							
estimated grazing	19.7 ± 4.3	20.6 ± 4.2	0.1 ± 0.1	38.8 ± 3.0	ns	***	ns
conserved forage	41.7 ± 3.2	41.4 ± 4.1	57.8 ± 1.4	26.4 ± 2.7	ns	***	*
grass silage	35.7 ± 3.3	36.9 ± 4.0	52.0 ± 2.0	21.7 ± 2.5	ns	***	ns
maize silage	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0			
cereal whole crop[b]	2.7 ± 1.0	3.6 ± 1.4	3.6 ± 1.5	2.8 ± 1.0	ns	ns	ns
straw/hay	3.4 ± 0.4	0.9 ± 0.3	2.2 ± 0.4	2.0 ± 0.5	*	ns	ns
concentrates	39.6 ± 1.8	40.0 ± 1.3	42.1 ± 1.4	37.7 ± 1.6	ns	*	*
supplements (kg/cow/day)							
mineral/vitamins	0.26 ± 0.05	0.10 ± 0.01	0.20 ± 0.05	0.15 ± 0.03	†	ns	ns
lipid[c]	0.15 ± 0.04	0.00 ± 0.00	0.05 ± 0.02	0.09 ± 0.04	†	ns	ns
health parameters (% of total cows)							
mastitis cases	1.6 ± 0.4	7.0 ± 0.6	5.0 ± 0.8	3.8 ± 0.7	**	†	ns
mastitis treatments	1.6 ± 0.4	6.2 ± 0.6	4.9 ± 0.8	3.1 ± 0.6	*	**	†
other health treatments	1.5 ± 0.4	2.5 ± 0.7	1.6 ± 0.4	2.3 ± 0.7	ns	ns	ns

[a]Significances were declared at ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; †, $0.05 < P < 0.10$ (trend); ns, $P > 0.10$ (nonsignificant). [b]Ensiled whole wheat plants (stem, leaves, and immature grain), harvested approximately 1 month before grain maturity. [c]Megalac, a commercial product consisting of extracted palm oil (mainly palmitic acid) and calcium.

were kept indoors), on the basis of farm records and questionnaires completed during regular farm visits (see Table 1 for the parameters recorded). Milk samples representing 24 h production were collected from the bulk tank after stirring and kept frozen at −20 °C until analysis. On each sampling date questionnaires were completed by the farmers recording the number of lactating cows, milk yield, feed composition and supplement use, housing and grazing management, herd health status (based on veterinary records), and recent calvings. Also, data on the gross composition of milk (fat, protein, lactose, and urea content and SCC) were collected from routine milk recording. This information, together with data on the genetics/breed composition of the herd, was used to define the husbandry/management practices. Estimated average DMI and grazing intake (by difference) were calculated as described by Butler et al.[7] using milk yield, breed, and feed composition data collected via the farmer questionnaire. Details of feeding regimens, milk yields, and gross composition and other farming system parameters are presented in Tables 1 and 2. The main characteristics of the four production systems in this study were as follows.

Organic Outdoor with Standard Milking Systems (OOS). Organic farms included were either certified by the Soil Association (three farms) or Organic Farmers and Growers (two farms), the two main U.K. certification bodies active in the North East of England. Animals were grazed during the outdoor season (typically between April and October) and kept indoors on high forage (usually grass silage diets) during the indoor season, with some farms providing occasional access to grazing swards during the indoor season. Swards used for grazing and silage production mostly consisted of mixed grass with white clover, and conservation swards for silage may also have included red clover. All organic farms included in the study calved all year round and used a variety of breeds including Holstein-Friesian (72%), Shorthorn (10%), Ayrshire (9%), Jersey (2%), British Friesian (4%), New Zealand Friesian (2%), and Meuse Rhine Issel (1%) cows (percentages in parentheses represent the average proportion among the five farms). Diets in organic farms were not fortified with vitamins (see Table 1).

Conventional Outdoor with Standard Milking Systems (COS). In conventional outdoor systems cows grazed on pasture during the grazing season and were kept indoors on diets consisting of preserved forage and concentrate with mineral and vitamin supplements during the indoor season. This represented the majority of dairy production in the United Kingdom. In contrast to the organic outdoor systems, farms used (a) mineral fertilized ryegrass dominated swards for grazing and silage production, (b) a lower proportion of forage in the diet throughout the year, (c) both vitamin and mineral feed supplements, and (d) mainly Holstein-Friesian cows (see Table 1). Similar to organic OOS farms, the COS farms used all year round calving.

Conventional Outdoor with Robotic Milking Systems (COR). Farms included used feeding, husbandry, and management regimens similar to those of the COS farms described under Conventional Outdoor with Standard Milking Systems (COS) above (Table 2), but had robotic milking systems.

Conventional Indoor with Standard Milking System (CIS). The farms included were representative/typical for such systems in the United Kingdom, with animals kept indoors all year round and fed a mixed diet of conserved forage (grass or grass/maize silage), relatively high levels of concentrate, and, occasionally, during the summer, freshly cut forage. The diet in such systems was relatively consistent throughout the year. Indoor systems had larger herds, greater concentrate, and lower fresh forage intake than both organic and conventional grazing-based dairy systems (see Organic Outdoor with Standard Milking Systems (OOS) and Conventional Outdoor with Standard Milking Systems (COS) above). An additional difference in management practice was that a proportion of animals were milked three times per day, especially during early lactation to maximize milk yield per cow. Farms used almost exclusively Holstein cows.

Chemicals and Analytical Standards. For the protein and FA profile analysis of milk samples, acetonitrile (>99.9%), trifluoroacetic acid (≥98%), guanidine hydrochloride, β-mercaptoethanol, rennet from *Mucor miehei*, type II, α-casein (αCN; ≥70%), β-casein (βCN; ≥90%),

κ-casein (κCN; ≥80%), α-lactalbumin (αL type I; ≥85% PAGE), β-lactoglobulinA (βLA; ≥90%), β-lactoglobulin B (βLB; ≥90%), bovine serum albumin (BSA; ≥98%), 37 FA methyl ester mix C4−24, t11 C18:1 and C22:5 c7,10,13,16,19 standards, potassium chloride (>99%), and trisodium citrate were purchased from Sigma-Aldrich (Gillingham, U.K.). Methanol (>99.8%), toluene (>99%), and acetyl chloride (>98%) were purchased from Fisher Scientific Ltd. (Loughborough, U.K.). Standards for conjugated linoleic acid (CLA) isomers c9t11 and t10c12 were kindly provided by colleagues in the Faculty of Agricultural Sciences, Aarhus University, Denmark. Chemicals and analytical standards used in milk α-tocopherol and carotenoid analyses are described in detail in the study of Slots et al.[10]

Milk Protein Analysis. The buffer composition for casein dilution was slightly modified compared to previous studies[19−21] and consisted of 6 M guanidine hydrochloride, 5 mM trisodium citrate, and 1% β-mercaptoethanol, adjusted to pH7 by either 1 M NaOH or 5 M NaOH solution. β-mercaptoethanol was used instead of dithiothreitol,[22] because it resulted in a better separation of milk caseins in our study. Milk was centrifuged at 14000 rpm for 15 min and the fat layer removed. Samples were then mixed and ultrasonicated for 30 min, before 20 μL of rennet, dissolved in ultrapure water (5 mg/mL), was added to 0.5 mL of the skimmed milk. Samples were placed in an incubator at 37 °C for 15 min and then centrifuged at 14000 rpm for 15 min. The upper liquid layer, corresponding to the whey proteins, was filtered through a 0.45 μm PVDF filter (Chromacol, U.K.) and transferred to a vial for immediate analysis by high-performance liquid chromatography (HPLC). Casein pellets were frozen, stored at −20 °C, freeze-dried overnight, and then dissolved in 1.5 mL of casein dilution buffer. Before transfer to HPLC vials for analysis, casein samples were filtered through a 0.45 μm PVDF filter. Protein analysis by HPLC was performed on a reversed-phase C4 analytical column (Alltech, Deerfield, IL, USA) with a 250 × 4.6 mm, 30 nm pore diameter and 5 μm particle size at 40 °C at a flow rate of 1 mL/min and an injection volume of 20 μL. Proteins were detected by UV absorbance at 214 nm. Mixed linear gradients and isocratic elutions were used for the separation of both caseins and whey proteins as described previously,[19−21] and buffer concentration was adjusted to optimize separation on the HPLC systems used.

Fractions of total casein separated and quantified by these methods were αCN (α_{s1}- and α_{s2}-caseins), βCN and κCN, whereas the whey protein fractions quantified were αL, BSA, βLA, and βLB.

Milk Fatty Acid Analysis. The milk sample preparation and gas chromatography analysis were described by Butler et al.[5] Quantification of individual FA was expressed as peak areas for each FA and as a proportion of total peak areas for all quantified FA. The total area of unidentified peaks (which may or may not have been fatty acid methyl esters) was <6.5% of total peak area. The individual FA allocated to different FA groups used in statistical analysis were as follows: (a) SFA C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, C18:0 (stearic acid, c18), C20:0, C22:0, C23:0, C24:0; (b) MUFA C14:1 c9, C16:1 c9, C18:1 t11 (vaccenic, VA), C18:1 c9 (oleic, OA), C20:1 c11; (c) PUFA C18:2 c9,12 (linoleic, LA), C18:3 c9,12,15 (α-linolenic, ALA), C20:3 c8,11,14, C20:4 c5,8,11,14, C22:2 c13,16, C20:5 c5,8,11,14,17 (eicosapentaenoic, EPA), C22:5 c7,10,13,16,19 (docosapentaenoic, DPA), CLA c9t11, CLA t10c12; (d) n-3 ALN, EPA, DPA; and (e) omega-6 (n-6) LA, C20:3 c8,11,14, C20:4 c5,8,11,14, CLA t10c12.

Milk Fat-Soluble Antioxidant Analysis. The fat-soluble antioxidant (carotenoids and α-tocopherol) concentrations in milk were analyzed by HPLC using the methods described by Slots et al.[10] One of the carotenoid peaks, which mainly represents canthaxanthin, may also contain other unidentified carotenoids and is therefore referred to in the results as "unknown carotenoids". Total carotenoids were calculated as the sum of lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene, and unknown carotenoids.

Statistical Analysis. Variables calculated as proportions (individual FA, SFA, MUFA, PUFA) were arcsine transformed, whereas other measurements were used untransformed. Descriptive statistics for all variables were carried out in untransformed data. Concentrations of proteins, FA, and antioxidants were expressed as grams per kilogram milk, grams per kilogram total identified milk FA, and milligrams per kilogram milk fat, respectively. Analyses of variance (ANOVA) were derived from linear mixed-effects models[23] using management type and season as fixed factors and farm as the random factor. Grazing season data were from samples collected in May, July, and August (when cows in the three outdoor systems were grazed outside), and indoor season data were from samples collected in December, February, and March (when cows were indoors in all four systems). Tukey's honest significant difference test was used for pairwise comparisons of means ($P < 0.05$). Analyses were performed in the R statistical environment,[24] and residual normality was assessed using the qqnorm function[25] with no data showing deviation from normality.

Multivariate redundancy analyses (RDA) were also carried out to relate individual feed components, robotic milking, and herd breed composition to (a) standard milk yield, milk quality, and animal health parameters (SCC, mastitis cases and treatments); (b) protein composition; (c) FA profiles; and (d) antioxidant (carotenoid and α-tocopherol) concentrations. Individual protein, FA profiles, and antioxidants were active variables in analyses, with totals derived from these data included as supplementary variables. Feed components used were dietary proportions of estimated grazing (GRA), concentrate feed (CON), grass or grass/clover silage (GS), maize silage (MS), cereal whole crop (WC; ensiled whole wheat plants, harvested approximately 1 month before grain maturity), and hay/straw (HS), as well as oil supplements (OS) and minerals and vitamins (VT). The proportion of Holstein-Friesian cows (HF) was used as a measure of herd breed composition, and robotic milking (RM) was a categorical variable. RDA analyses were performed using the CANOCO package,[26] using automatic forward selection of variables with their significance calculated using Monte Carlo permutation tests.

RESULTS

Milk Yield and Basic Composition and Animal Health Parameters. When the performances of the three dairy systems with standard milking parlors, but contrasting feeding intensities (organic outdoor, OOS; conventional outdoor, COS; and conventional indoors, CIS) were compared, significant differences in total milk fat and protein content, but not in total milk yield and urea concentrations, could be detected between dairy systems and/or season (Table 1). Fat content was higher in the indoor season, but there was no significant effect of dairy systems. Protein content was higher in the two outdoor systems than in the indoor system and higher during the grazing season. There was a trend toward differences in milk yield, with the indoor system recording 18 and 7% higher yields than the organic and conventional outdoor systems, respectively (Table 1).

When the performances of the two outdoor conventional dairy systems with different milking parlors (farms with standard milking parlors, COS vs farms with robotic milking systems, COR) were compared, significant differences in total milk fat and protein contents, but not total milk yield and urea concentrations, could be detected between dairy systems and/or milking season (Table 2). Fat content was higher in the indoor milking season, but there was no significant effect of dairy systems. Protein content was higher in milk from farms using standard milking parlors and higher during the grazing season.

No significant difference in animal health could be detected between the three dairy systems using standard milking parlors but contrasting feeding intensity (Table 1). In contrast, when the two outdoor conventional dairy systems with different milking parlors were compared, a significantly (>3 times) higher proportion of clinical mastitis cases and mastitis treatments were recorded in farms using robotic milking systems. However, it should be pointed out that there was considerable variation in mastitis incidence between farms using robotic milking. Also, a higher proportion of cows were treated for mastitis during the indoor season (Table 2). However, SCC in milk (an indicator of

subclinical mastitis) were not significantly different between seasons and farms using contrasting milking systems.

When the relationships between dairy breed choice, milking system and feed components, and udder health parameters (cases of clinical mastitis and SCC) were investigated by RDA (Figure 1), positive associations were detected between (a) SCC and the use of Holstein-Friesian cows along axis 1 and (b) mastitis cases and treatments and robotic milking. In contrast, there were negative associations between (a) SCC and cereal whole crop along axis 1 and (b) mastitis cases and treatments and vitamin supplements along axis 2 (Figure 1).

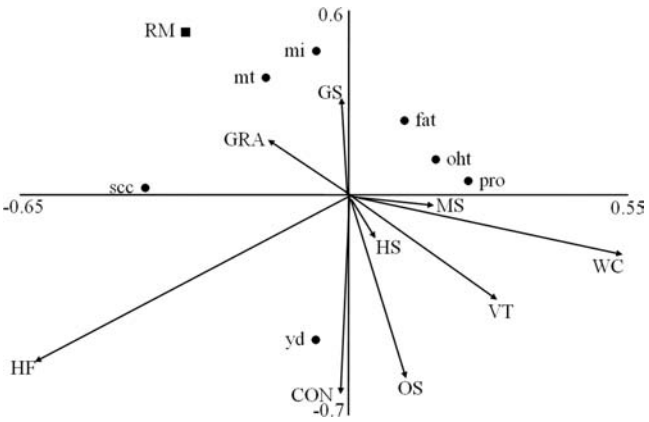

Figure 1. Biplot derived from the redundancy analysis showing the relationship between standard milk yield and quality and animal health parameters (fat = fat content, pro = protein content, scc = somatic cell count, yd = yield, mi = mastitis incidences, mt = mastitis treatments, oht = other health treatments, all shown as ●) and production system variables. Continuous variables (shown as arrows): HF = proportion of Holstein-Friesian cows in the herd ($F = 7.75$, $P = 0.006$); WC = cereal whole crop ($F = 5.28$, $P = 0.016$); VT = vitamin and mineral supplements ($F = 3.61$, $P = 0.058$); MS = maize silage ($F = 2.43$, $P = 0.116$); HS = hay/straw ($F = 0.38$, $P = 0.544$); GRA = grazing ($F = 0.22$, $P = 0.678$); GS = grass silage ($F = 0.12$, $P = 0.772$); OS = oil supplements ($F = 0.37$, $P = 0.534$); CON = concentrate (collinear); categorical variable (■): RM = robotic milking ($F = 0.77$, $P = 0.396$). Axis 1 explained 15.8% of the variation and axis 2 a further 0.3%.

Protein Composition. When the performances of the three dairy systems with standard milking parlors, but contrasting feeding intensities, were compared (OOS, COS, CIS), a significant main effect of production system could be detected only for total β-lactoglobulin (tβL; sum of βLA and βLB), which was found in higher (8%) concentrations in milk from the two outdoor systems (OOS, COS) than from the indoor system (CIS) (Table 3). However, milking season also had a significant effect on several protein groups, with higher concentrations of total casein, β- and κCN, and βLA and a higher casein/whey ratio being detected in milk during the indoor season (Table 3).

When the performances of the two outdoor conventional dairy systems with different milking parlors (standard milking parlors, COS; robotic milking systems, COR) were compared, a significant main effect of production system and season could be detected only for tβL, which was found in higher (5%) concentrations in COS milk and during the indoor season (Table 4).

Results from the RDA showed that total casein concentrations were positively associated with cereal whole crop inclusion in the diets and negatively associated with the use of Holstein-Friesian cows along axis 1 (Figure 2). Concentrations of total protein, αCN, βCN, and κCN were positively associated with cereal whole crop in the diet and negatively associated with (a) the proportions of Holstein-Friesian cows in the herd, (b) concentrate and fat supplement use, and (c) robotic milking (Figure 2). However, there were no associations between whey protein concentrations and feed and breed drivers (Figure 2).

Fatty Acid Composition. When the performances of the three dairy systems with standard milking parlors, but contrasting feeding intensities (OOS, COS, CIS), were compared, significant main effects of production system could be detected only for

Table 3. Main Effect Means ± SE and ANOVA *P* Values for the Effect of Feeding Intensity (OOS, Organic Outdoors; COS, Conventional Outdoors; CIS, Conventional Indoors) and Season (Indoor, December, February, March; Grazing, May, June, July) on the Protein Composition (Grams per Kilogram Milk) of Milk from Dairy Farms in the North East of England

	feeding intensity (F)			season (S)		ANOVA *P* values[a]		
parameter assessed	OOS ($n = 29$)	COS ($n = 28$)	CIS ($n = 30$)	indoor ($n = 58$)	grazing ($n = 59$)	F	S	F × S
protein groups								
total protein	37.4 ± 0.4	37.0 ± 0.5	36.5 ± 0.4	37.6 ± 0.3	36.3 ± 0.4	ns	**	ns
casein	31.8 ± 0.4	31.3 ± 0.4	31.1 ± 0.4	32.1 ± 0.3	30.7 ± 0.3	ns	**	ns
whey protein	5.7 ± 0.1	5.7 ± 0.1	5.4 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	ns	ns	ns
ratio casein/whey	5.6 ± 0.1	5.6 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.6 ± 0.1	ns	*	ns
caseins								
α-casein	12.4 ± 0.1	12.3 ± 0.1	12.0 ± 0.1	12.3 ± 0.1	12.2 ± 0.1	ns	ns	ns
β-casein	13.0 ± 0.1	12.7 ± 0.2	12.9 ± 0.2	13.3 ± 0.1	12.5 ± 0.1	ns	***	ns
κ-casein	6.3 ± 0.1	6.3 ± 0.1	6.2 ± 0.1	6.5 ± 0.1	6.1 ± 0.1	ns	***	ns
whey proteins								
α-lactalbumin	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	ns	†	ns
bovine serum albumin	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	ns	ns	ns
β-lactoglobulin A	2.2 ± 0.1	2.2 ± 0.1	2.0 ± 0.0	2.2 ± 0.1	2.1 ± 0.0	ns	*	ns
β-lactoglobulin B	2.1 ± 0.1	2.0 ± 0.1	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	ns	ns	ns
total β-lactoglobulin	4.2 ± 0.1 a	4.2 ± 0.1 a	3.9 ± 0.1 b	4.2 ± 0.0	4.1 ± 0.1	*	ns	ns

[a]Significances were declared at ***, $P < 0.001$; **, $P < 0.01$; *, $0.01 < P < 0.05$; †, $0.05 < P < 0.10$ (trend); ns, $P > 0.10$ (nonsignificant). Means for systems with different feeding intensities within rows and with different letters are significantly different ($P < 0.05$) according to Tukey's honestly significant difference test.

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Table 4. Main Effect Means ± SE for the Effects of Robotic Milking System (COS, Standard Milking Parlors; COR, Robotic Milking Parlors) and Season (Indoor, December, February, March; Grazing, May, June, July) on Milk Protein (Grams per Kilogram Milk), Fat (Grams per Kilogram Total Fatty Acids), and Antioxidants (Milligrams per Kilogram Fat) on Farms of Similar Feeding Intensities in the North East of England[a]

	milking system (M)		season (S)		ANOVA P values[b]		
parameter assessed	COS (n = 28)	COR (n = 30)	indoor (n = 28)	grazing (n = 30)	M	S	M × S
proteins							
β-lactoglobulin	4.2 ± 0.1	4.0 ± 0.1	4.2 ± 0.0	4.0 ± 0.1	*	*	ns
fatty acids (% of total FA)							
PUFA[c]	34.6 ± 1.0	30.1 ± 0.8	30.1 ± 0.9	34.3 ± 0.9	†	*	ns
C12:0	34.9 ± 1.2	40.8 ± 1.1	39.7 ± 1.2	36.4 ± 1.2	*	*	ns
C14:0	113 ± 2	121 ± 2	119 ± 2	115 ± 3	†	ns	ns
RA	6.9 ± 0.5	5.3 ± 0.4	4.4 ± 0.3	7.6 ± 0.4	†	*	ns
antioxidants							
β-cryptoxanthin	0.09 ± 0.01	0.07 ± 0.00	0.08 ± 0.01	0.07 ± 0.01	†	ns	ns

[a]Only parameters for which significant main effects or interactions were detected are shown. [b]Significances were declared at ***, $P < 0.001$; **, $P < 0.01$; *, $0.01 < P < 0.05$; †, $0.05 < P < 0.10$ (trend); ns, $P > 0.10$ (nonsignificant). [c]PUFA: C18:2 c9c12 (LA); C18:3 c9c12c15 (ALA); C18:2 c9t11 (RA); C18:2 t10c12; C20:3 c8c11c14; C20:4 c5c8c11c14; C20:5 c5c8c11c14c17; C22:2 c13c16; C22:5 c7c10c13c16c19.

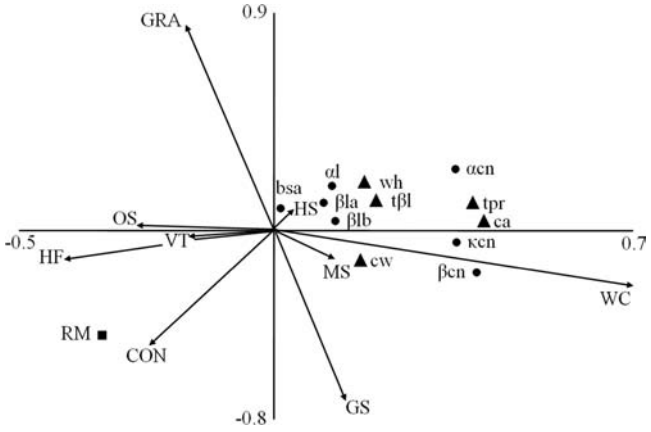

Figure 2. Biplot derived from the redundancy analysis showing the relationship between milk protein composition (tpr = total protein, ca = casein, wh = whey protein, cw = casein/whey protein, tβl = total β-lactoglobulin, all shown as ▲; αcn = α-casein, βcn = β-casein, κcn = κ-casein, αl = α-lactalbumin, bsa = bovine serum albumin, βla = β-lactoglobulin A, βlb = β-lactoglobulin B, all shown as ●) and production system variables. Continuous variables (shown as arrows): WC = cereal whole crop (F = 7.71, P = 0.004); CON = concentrate (F = 3.09, P = 0.062); GRA = grazing (F = 2.24, P = 0.109); OS = oil supplements (F = 1.49, P = 0.202); HS = hay/straw (F = 1.46, P = 0.202); HF = proportion of Holstein-Friesian cows in the herd (F = 1.34, P = 0.230); GS = grass silage (F = 0.94, P = 0.366); VT = vitamin and mineral supplements (F = 0.44, P = 0.632); MS = maize silage (collinear). Categorical variable (■): RM = robotic milking (F = 2.04, P = 0.136). Axis 1 explained 23.2% of the variation and axis 2 a further 4.9%.

concentrations of total n-3, ALA, EPA, and DPA and the n-3:n-6 ratio, which were all found to decrease with increasing feeding intensity (OOS < COS < CIS) (Table 5). In contrast, milking season affected a large number of fat composition parameters. Concentrations of total SFA, palmitic acid, total n-6, and LA were higher in the indoor season. In contrast, concentrations of total MUFA and PUFA and of stearic acid, OA, VA, ALA, EPA, and RA and the n-3:n-6 ratio were higher in the grazing season.

Significant interactions between production systems and season were detected for SFA, MUFA, n-3, the n-3:n-6 ratio, palmitic acid, OA, VA, ALA, and RA (Table 5). With increasing feeding intensity (OOC < COS < CIS) SFA concentrations decreased during the indoor season, but increased during the grazing season, whereas the opposite trend was detected for MUFA concentrations (Figure 3). Concentrations of n-3 and ALA and the n-3:n-6 ratio increased with decreasing feeding intensity in both seasons, but the relative difference between systems was greater during the grazing season. Also, significant differences between the outdoor (COS) and indoor (CIS) conventional systems for n-3 and ALA were detected only during the grazing season (Figure 3). For palmitic acid, VA and RA significant differences between production systems were detected only during the grazing season, with VA and RA concentrations decreasing and palmitic acid concentrations increasing with increasing feeding intensity (OOS < COS < CIS) (Figure 3).

When the performances of the two outdoor conventional dairy systems with different milking parlors (standard milking parlors, COS; robotic milking systems, COR) were compared, a significant main effect of the production systems could be detected only for lauric acid, with higher concentration being detected in milk from robotic milking farms (Table 4). There were also trends toward higher concentrations of PUFA and RA and lower concentrations of myristic acid in milk from farms using standard milking parlors (COS). Significant differences in fat composition found between the grazing and indoor season followed trends similar to those reported for the comparison of OOS, COS, and CIS farms (see above), with PUFA and RA concentration being higher in the grazing season and concentrations of lauric acid being higher during the indoor season (Table 4).

RDA revealed positive associations between concentrations of total MUFA, n-3, RA, VA, stearic acid, OA, and ALA (and to a lesser extent EPA and DPA) and the n-3:n-6 ratio with grazing, along axis 1 (Figure 4). In contrast, total SFA, n-6, LA, and palmitic acid concentrations were positively associated with grass silage, total conserved forage, cereal whole crop, and concentrate and oil supplement intake (and to a lesser extent the use of Holstein-Friesian cows) along axis 1. There was also a positive association between total PUFA concentrations and hay/straw intake along axis 2 (Figure 4).

Fat-Soluble Antioxidant Composition. When the performances of the three dairy systems with standard milking parlors, but contrasting feeding intensities (OOS, COS, CIS), were compared, significant main effects of the production systems could be detected for total carotenoids and most

Table 5. Main Effect Means ± SE and ANOVA *P* Values for the Effect of Feeding Intensity (OOS, Organic Outdoors; COS, Conventional Outdoors; CIS, Conventional Indoors) and Season (Indoor; December, February, March; Grazing, May, June, July) on the Fatty Acid Composition (Grams per Kilogram Total Fatty Acids) of Milk from Dairy Farms in the North East of England

	feeding intensity (F)			season (S)		ANOVA *P* values[a]		
parameter assessed	OOS (n = 29)	COS (n = 28)	CIS (n = 30)	indoor (n = 58)	grazing (n = 59)	F	S	F × S
fatty acid groups								
SFA[b]	705 ± 7	702 ± 5	711 ± 6	721 ± 3	691 ± 5	ns	***	**
MUFA[c]	258 ± 6	263 ± 5	252 ± 5	244 ± 3	271 ± 5	ns	***	**
PUFA[d]	36.4 ± 1.1	34.6 ± 1.0	37.6 ± 1.2	34.5 ± 0.9	38.0 ± 0.9	ns	***	ns
n-3[e]	10.0 ± 0.5 a	6.2 ± 0.2 b	5.0 ± 0.2 b	6.3 ± 0.3	7.8 ± 0.5	***	***	***
n-6[f]	19.0 ± 0.8	21.6 ± 1.0	27.2 ± 1.3	23.3 ± 0.9	22.0 ± 1.0	†	*	ns
n-3:n-6	0.55 ± 0.04 a	0.31 ± 0.02 b	0.20 ± 0.02 b	0.30 ± 0.02	0.41 ± 0.04	**	***	***
SFA								
C14:0	121 ± 2	113 ± 2	111 ± 2	117 ± 1	113 ± 2	ns	ns	ns
C16:0	328 ± 7	352 ± 60	362 ± 9	360 ± 5	335 ± 7	ns	***	***
C18:0	121 ± 3	114 ± 3	117 ± 5	114 ± 3	120 ± 3	ns	*	ns
MUFA								
OA	212 ± 5	217 ± 4	210 ± 5	204 ± 3	223 ± 4	ns	***	*
VA	16.3 ± 1.4	13.4 ± 1.1	10.9 ± 1.0	9.9 ± 0.5	17.0 ± 1.1	†	***	***
PUFA								
ALA	8.6 ± 0.4 a	5.1 ± 0.2 b	4.2 ± 0.2 b	5.2 ± 0.3	6.6 ± 0.4	***	***	***
EPA	0.6 ± 0.1 a	0.5 ± 0.0 ab	0.4 ± 0.0 b	0.5 ± 0.03	0.6 ± 0.04	*	**	ns
DPA	0.7 ± 0.1 a	0.5 ± 0.0 ab	0.4 ± 0.0 b	0.6 ± 0.06	0.6 ± 0.03	*	ns	ns
LA	16.7 ± 0.7	19.1 ± 0.9	24.1 ± 1.2	20.7 ± 0.9	19.4 ± 1.0	†	*	ns
RA	7.4 ± 0.6	6.9 ± 0.5	5.5 ± 0.5	5.0 ± 0.2	8.2 ± 0.5	ns	***	**

[a]Significances were declared at ***, $P < 0.001$; **, $P < 0.01$; *, $0.01 < P < 0.05$; †, $0.05 < P < 0.10$ (trend); ns, $P > 0.10$ (nonsignificant). Means for systems with different feeding intensities within rows and with different letters are significantly different ($P < 0.05$) according to Tukey's honestly significant difference test. [b]SFA, C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, C20:0, C22:0, C24:0. [c]MUFA: C14:1 c9, C16:1 c9, C18:1 c9 (OA), C18:1 t11 (VA), C20:1 c11. [d]PUFA: n-3, n-6, C18:2 c9t11 (RA). [e]n-3 FA: C18:3 c9c12c15 (ALA), C20:5 c5c8c11c14c17 (EPA), C22:5 c7c10c13c16c19 (DPA). [f]n-6 FA: C18:2 c9c12 (LA), C20:3 c8c11c14, C20:4 c5c8c11c14, C22:2 c13c16, C18:2 t10c12.

individual carotenoids, but not for α-tocopherol (Table 6). Carotenoid concentrations were significantly higher in milk from the two outdoor systems (OOS and COS) than from the indoor system, but no significant differences were detected between the two outdoor systems except for zeaxanthin, which was found in higher concentrations in OOS compared to COS milk (Table 6). Season had a significant main effect on all carotenoids (except for β-cryptoxanthin) and α-tocopherol (Table 6). Concentrations of carotenoids were higher during the grazing season, whereas concentrations of α-tocopherol were higher during the indoor season (Table 6).

Significant interactions between production systems and season were detected for lutein, α-carotene, and a group of unknown carotenoids (Table 6). Relative differences between the two outdoor dairy systems (OOS and COS) and the indoor (CIS) dairy system were greater during the grazing than the indoor season (Figure 5).

When the performances of the two outdoor conventional dairy systems with different milking parlors (COS and COR) were compared, no significant main effects of production system and season could be detected on the concentrations of fat-soluble antioxidants in milk (Table 4).

RDA showed that concentrations of total carotenoids, α- and β-carotene, lutein, and zeaxanthin were positively associated with grazing intake along axis 2, but negatively associated with cereal whole crop, maize silage, concentrate and oil supplement in the dairy diet, and the use Holstein-Friesian cows along both axes 1 and 2 (Figure 6). Also, β-cryptoxanthin, unknown carotenoids, and α-tocopherol were positively associated with the grass silage and hay/straw in the diet and vitamin supplementation (Figure 6).

DISCUSSION

Here we report for the first time the effects of production system intensification (especially reduced grazing and increased use of concentrate/conserved forage in dairy diets and robotic milking) on both (a) standard milk yield and quality and animal health parameters and (b) a wide range of nutritionally relevant milk quality parameters (protein composition, FA profiles, and concentrations of fat-soluble antioxidants) by comparing milk from contrasting commercial dairy production systems in the North East of England.

Milk Yield and Basic Composition and Animal Health Parameters. As expected from previous studies,[7] organic farms had the lowest numerical milk yield and milk from outdoor systems had a higher total protein content. However, surprisingly there was only a trend toward feeding system intensification having a significant impact on milk yield.[11] Also, robotic milking resulted in a slight (5%) numerical decrease in milk yield per cow, even though it is known to increase milking frequency and would therefore have been expected to increase milk yield. This may have been linked to the higher incidence of clinical mastitis and mastitis treatments recorded in COR farms. This confirms previous studies reporting an increase in mastitis incidence in farms with robotic milking systems.[27] This is of concern from an animal welfare point of view, but may also increase the risk of transferable antibiotic resistance being introduced into cattle and potential human pathogens (e.g., *Escherichia coli*).[28] It may also increase veterinary costs and thereby reduce farm business viability.

Protein Composition. Feeding system intensification and the use of robotic milking had a relatively limited effect of the milk protein composition parameters investigated. The only

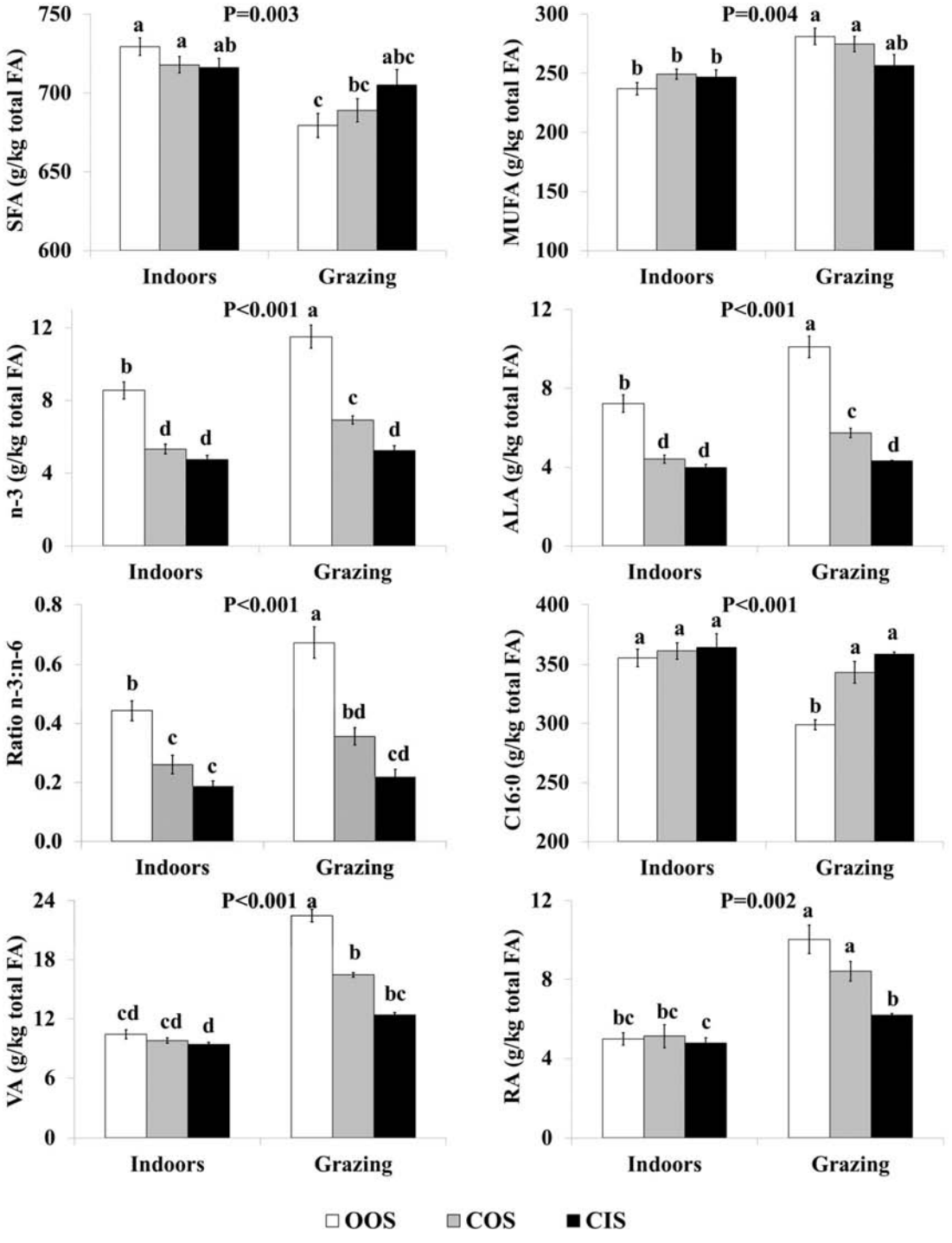

Figure 3. Interaction means ± SE for the effects of feeding intensity (OOS, organic outdoors; COS, conventional outdoors; CIS, conventional indoors) and season (indoor vs grazing season) on the concentrations of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), omega-3 fatty acids (n-3), α-linolenic acid (ALA), palmitic acid (C16:0), vaccenic acid (VA), and rumenic acid (RA) and the n-3:n-6 ratio of milk from dairy farms in northeastern England. *P* represents the ANOVA *P* value for the interaction. Bars labeled with the same letter are not significantly different (Tukey's honestly significant difference test, $P < 0.05$).

protein affected by both feeding and milking system intensification was tβL. This finding is of nutritional relevance because in humans, dietary tβL intake was shown to regulate phosphorus metabolism in the mammary gland and to improve immunity in newborns.[29] It was also described as a good source of cysteine, which contributes to muscle growth, and the bioactive peptides released from tβL after gastrointestinal digestion were shown to have antihypertension, antioxidant, antimicrobial, immunomodulating, and hypocholesterolemic properties.[29,30] The concentrations of tβL were lower in milk from farms that had intensified production by year-round indoor production or robotic milking. These results agree with previous studies which reported that concentrations/composition of whey proteins are affected by dairy diets and that is possible to increase milk tβL content of milk by increasing fresh forage intake.[13] The higher fresh grass intake by cows in outdoor grazing farms (OOS

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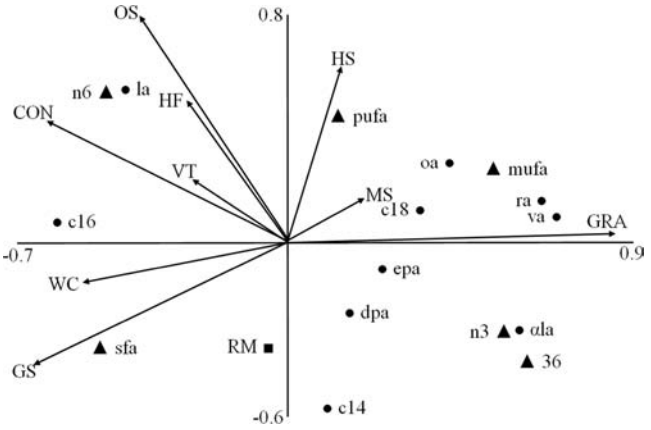

Figure 4. Biplot derived from the redundancy analysis showing the relationship between milk fatty acid profile (sfa = saturated fatty acids, mufa = monounsaturated fatty acids, pufa = polyunsaturated fatty acids, n3 = omega-3 fatty acids, n6 = omega-6 fatty acids, 36 = n-3:n-6 ratio, all shown as ▲; c14 = myristic acid, c16 = palmitic acid, c18 = stearic acid, va = vaccenic acid, oa = oleic acid, la = linoleic acid, αla = α-linolenic acid, ra = rumenic acid, epa = eicosapentaenoic acid, dpa = docosapentaenoic acid, all shown as ●) and production system variables. Continuous variables (shown as arrows): GRA = grazing ($F = 31.29$, $P = 0.002$); OS = oil supplements ($F = 6.28$, $P = 0.010$); MS = maize silage ($F = 4.85$, $P = 0.010$); HS = hay/straw ($F = 4.23$, $P = 0.020$); HF = proportion of Holstein-Friesian cows in the herd ($F = 1.37$, $P = 0.256$); GS = grass silage ($F = 0.41$, $P = 0.590$); WC = cereal whole crop ($F = 0.39$, $P = 0.658$); VT = vitamin and mineral supplements ($F = 0.14$, $P = 0.884$); CON = concentrate (collinear). Categorical variable (■): RM = robotic milking ($F = 0.74$, $P = 0.428$). Axis 1 explained 27.9% of the variation and axis 2 a further 4.2%.

and COS) is therefore the most likely explanation for the increased tβL concentrations in milk compared to milk from the indoor system. Robotic milking also resulted in lower tβL content. The frequency of milking was not assessed in the study presented here, but previous work reported an average milking frequency of 2.4−2.6 times per day for robotic milking systems.[11] Thus, the higher milking frequency associated with robotic milking and the indoor production systems (where cows were milked three times per day during early lactation) may also have contributed to the differences in milk protein content/composition, as suggested by Klei et al.[14] However, lower tβL concentrations may also have been related to the higher mastitis incidence in robotic milking farms, because milk from cows with clinical mastitis was previously shown to have lower tβL concentrations.[31] Apart from being a nutritionally desirable compound, high concentrations of tβL in milk were reported to improve efficiency in cheesemaking (cheese yield per kilogram of milk).[32]

The significant dietary increase (34%) of cereal whole crop, which was positively associated with concentrations of total casein, βCN, κCN, and the casein/whey protein ratio in the RDA, during the indoor season can be the main factor resulting in their increase in milk, compared with the grazing season milk. RDA indicated an effect of dairy cattle breed on milk casein (α-, β-, and κ-caseins) and tβL concentrations, which were all negatively associated with the proportion of Holstein-Friesian cows in the herd. Previous studies reported lower protein concentrations (both whey and casein) and casein/protein ratios in milk from Holstein-Friesian compared with Jersey cows.[16] It would therefore be of interest to evaluate the impact of breed choice and potential breed × production system interactions in more detail by including a larger sample of both organic and conventional farms with non-Holstein-Friesian cows in future studies.

Fatty Acid Composition. As expected from previous studies into the effect of dairy nutrition on FA profiles of milk,[5,7,33] feeding system intensification had a significant impact on concentrations of nutritionally relevant FA in milk. In addition, in this study ANOVA also identified some effects of robotic milking on FA profiles, and results from the RDA identified associations between breed composition and FA profiles.

Most importantly, the study confirmed previous reports that extensive feeding regimens with a high grazing-based, fresh forage intake increase (a) concentrations of nutritionally desirable PUFA (ALA, EPA, DPA, RA) and MUFA (VA) and (b) the n-3:n-6 ratio in milk.[7,34] This is thought to be mainly because fresh forage is high in PUFA, especially in ALA,[35] some of which is biohydrogenated to VA in rumen and subsequently transformed to RA in the mammary gland.[36] As well as the direct positive effects of grazing on dietary PUFA supply and concentrations in milk, some studies suggested that high-forage diets, with grass, silage, or straw/hay instead of concentrates, increase the production of intermediates of PUFA biohydrogenation, such as VA and RA, in the rumen.[37] In systems where cows have access to pasture during grazing season (OOS, COS)

Table 6. Main Effect Means ± SE and ANOVA *P* Values for the Effect of Feeding Intensity (OOS, Organic Outdoors; COS, Conventional Outdoors; CIS, Conventional Indoors) and Season (Indoor, December, February, March; Grazing, May, June, July) on the Antioxidant Composition (Milligrams per Kilogram Fat) of Milk from Dairy Farms in the North East of England

	feeding intensity (F)			season (S)		ANOVA *P* values[a]		
parameter assessed	OOS ($n = 29$)	COS ($n = 28$)	CIS ($n = 30$)	indoor ($n = 58$)	grazing ($n = 59$)	F	S	F × S
lutein	0.47 ± 0.03 a	0.44 ± 0.02 a	0.26 ± 0.02 b	0.31 ± 0.01	0.46 ± 0.03	***	***	***
zeaxanthin	0.06 ± 0.00 a	0.05 ± 0.00 b	0.03 ± 0.00 c	0.04 ± 0.00	0.05 ± 0.00	***	***	†
β-cryptoxanthin	0.08 ± 0.04 a	0.09 ± 0.04 a	0.05 ± 0.03 b	0.08 ± 0.00	0.07 ± 0.01	**	ns	ns
α-carotene	0.02 ± 0.04 a	0.02 ± 0.06 a	0.01 ± 0.04 b	0.01 ± 0.00	0.02 ± 0.00	**	***	*
β-carotene[b]	6.62 ± 0.20 a	6.78 ± 0.26 a	4.57 ± 0.23 b	5.51 ± 0.23	6.40 ± 0.24	**	*	ns
unknown carotenoids	0.23 ± 0.16	0.25 ± 0.21	0.22 ± 0.20	0.33 ± 0.02	0.13 ± 0.02	ns	***	***
total carotenoids[c]	7.48 ± 0.22 a	7.62 ± 0.28 a	5.14 ± 0.27 b	6.28 ± 0.26	7.15 ± 0.26	**	***	ns
α-tocopherol	18.4 ± 0.9	18.1 ± 0.6	16.3 ± 0.6	19.3 ± 0.6	16.0 ± 0.4	ns	***	ns

[a]Significances were declared at ***, $P < 0.001$; **, $P < 0.01$; *, $0.01 < P < 0.05$; †, $0.05 < P < 0.10$ (trend); ns, $P > 0.10$ (nonsignificant). Means for systems with different feeding intensities within rows and with different letters are significantly different ($P < 0.05$) according to Tukey's honestly significant difference test. [b]β-Carotene refers to the summary of all *cis*- and *trans*-β-carotene isomers. [c]Total carotenoids: lutein + zeaxanthin + β-cryptoxanthin + α-carotene + β-carotene + unknown carotenoids.

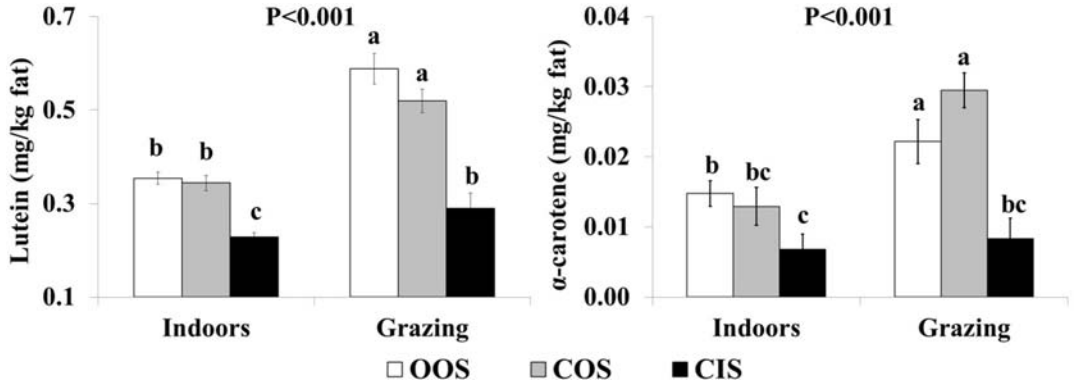

Figure 5. Interaction means ± SE for the effects of feeding intensity (OOS, organic outdoors; COS, conventional outdoors; CIS, conventional indoors) and season (indoor vs grazing season) on the lutein and α-carotene concentrations in milk from dairy farms in the North East of England. *P* represents the ANOVA *P* value for the interaction. Bars labeled with the same letter are not significantly different (Tukey's honestly significant difference test, $P < 0.05$).

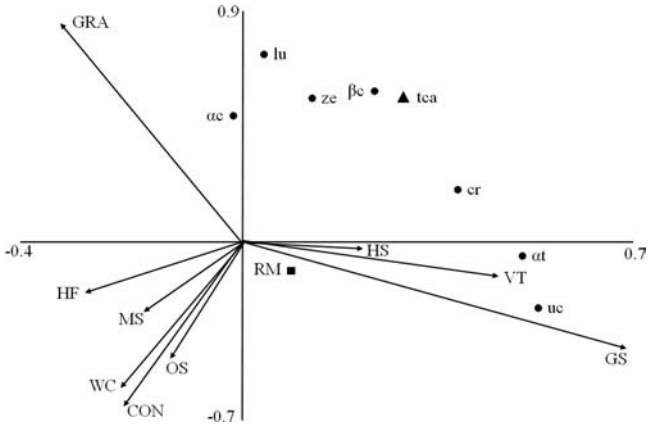

Figure 6. Biplot derived from the redundancy analysis showing the relationship between milk antioxidant concentrations (tca = total carotenoids shown as ▲; lu = lutein, ze = zeaxanthin, cr = β-cryptoxanthin, αc = α-carotene, βc = β-carotene, uc = unknown carotenoids, αt = α-tocopherol, all shown as ●) and production system variables. Continuous variables (shown as arrows): GS = grass silage ($F = 15.33$, $P = 0.002$); GRA = grazing ($F = 7.83$, $P = 0.002$); VT = vitamin and mineral supplements ($F = 6.59$, $P = 0.016$); HF = proportion of Holstein-Friesian cows in the herd ($F = 3.23$, $P = 0.066$); HS = hay/straw ($F = 3.05$, $P = 0.078$); WC = cereal whole crop ($F = 1.68$, $P = 0.190$); OS = oil supplements ($F = 0.41$, $P = 0.524$); MS = maize silage ($F = 0.34$, $P = 0.646$); CON = concentrate (collinear). Categorical variable (■): RM = robotic milking ($F = 0.53$, $P = 0.520$). Axis 1 explained 23.2% of the variation and axis 2 a further 4.9%.

milk SFA concentrations were decreased when cows were grazing, as a result of increased fresh grass intake,[7] whereas in CIS farms, where the diet was consistent throughout the year, SFA content was not affected by season. Results from the RDA indicate that the association between grazing and n-3 concentrations was not as strong as that between grazing and RA and VA concentrations in milk. This may explain why differences in n-3 (but not RA) between organic and conventional milk remain significant in winter,[5] when organic herds switch to diets high in conserved forage, whereas higher levels of concentrate are used in conventional systems. Other recent studies concluded the increased concentrations of n-3 in organic milk were at least partially caused by the inclusion of clover in grazing and conservation swards[38] instead of pure grass swards used in farms under conventional management. The use of clover silage has been shown to increase ALA concentrations in milk.[39]

On the other hand, results from the RDA indicate that diets rich in concentrates and maize silage with low fresh grass intake (those used in the conventional indoor farms) result in higher concentrations of total n-6 and LA (the main n-6 found in milk). This confirms previous studies reporting higher LA concentrations in milk when high levels of concentrates, maize byproduct, and silage are used instead of grass silage.[10,40,41]

An increase in dietary intake of n-3 (in particular, long-chain n-3 such as EPA and DPA) was linked to reduced risk of certain cancers and CVD.[42] Similar benefits were reported for CLA, but evidence is mainly from animal studies.[6,8] Although LA and other n-6 are also essential FA, increasing n-6 intake in the human diet is less desirable than increasing n-3 intake.[42] The n-3:n:6 ratio in northwestern European and North American diets is typically between 1:15 and 1:16.7. This is considered to be too low because a high intake of n-6 promotes CVD, cancer, and inflammatory and autoimmune diseases.[42] It has been recommended that the n-3:n-6 ratio in the human diet should be between 1:4 and 1:1.[42] From a human nutrition point of view an increase in n-3, MUFA, and RA concentrations and the n-3:n-6 ratio in milk is therefore considered to be desirable.[3]

The finding of very limited effects of feeding regimen intensification on concentrations of undesirable SFA confirms results from previous studies that compared milk from organic and conventional systems with contrasting feeding intensities.[5,7] However, RDA identified positive associations between the proportion of non-Holstein-Friesian cows in the herd and concentrations of lauric acid and myristic acid (C14:0). Also, previous studies reported that milk from Jersey cows has a higher SFA concentration than milk from Holstein-Friesian cows.[43] The effect of breed choice, breeding systems, and/or herd breed composition on FA profiles should therefore be further investigated in more detail in the future.

Antioxidant Composition. As expected from previous studies into the effect of dairy nutrition on antioxidant/vitamin concentrations in milk,[7,44] feeding system intensification had a negative effect on concentrations of nutritionally relevant carotenoids in milk. Most importantly, vitamin supplementation in the conventional indoor system was not able to compensate for the intake of antioxidants via grazing in the outdoor systems. However, there were very limited effects of robotic milking on antioxidant levels. Results from the RDA indicate that other parameters related to production system intensity (in particular, vitamin supplementation and use of Holstein-Friesian cows) may

also affect the carotenoid and α-tocopherol contents of milk. However, in contrast to other U.K. surveys, the difference in antioxidant concentrations between outdoor organic and conventional systems using standard milking parlors was not significant.[7] Results from the RDA indicate that this may have been due to the vitamin supplementation (which is not permitted under organic farming standards) in conventional outdoor systems having compensated for the lower forage-associated vitamin intake. The higher concentrations of α-tocopherol in milk during the indoor season can be an outcome of the numerically increased (24%) dietary vitamin supplementation compared with the grazing season. RDA also indicated that the lower antioxidant concentrations in milk from indoor conventional farms is primarily due to the proportion of concentrate and conserved forage (including maize silage and whole crop cereals) in the diet. This agrees with a review of Noziere et al.,[17] who reported that the carotenoid content of maize silage and concentrate feeds is relatively low.

Higher dietary antioxidant intake (especially α-tocopherol) has been shown to contribute to cows' health status by protecting against mastitis and enhancing the immune system.[45,46] This is confirmed by the RDA, which identified negative associations between mastitis incidence and vitamin supplementation. In humans, an increased antioxidant intake has been linked to a reduced risk of CVD, oxidative stress, and certain cancers.[1,7] Also, the fat-soluble antioxidants in milk reduce the oxidation of lipids[47] and proteins[48] and improve the shelf life of milk.[44] Milk with increased antioxidant content may therefore provide benefits to dairy cows, milk processors, retailers, and consumers.

The study reported here provides new evidence for the hypothesis that the intensification of U.K. dairy production has resulted in a reduction in the concentrations of nutritionally desirable compounds (proteins, FA, and antioxidants) in milk. In addition, robotic milking was shown to increase the incidence of clinical mastitis and veterinary antibiotic use. Although milk from more extensive organic and conventional outdoor systems was shown to have higher concentrations of certain nutritionally desirable proteins, FA, and antioxidants, compared with milk from more intensive systems, it is not possible to predict the potential human health benefits of consuming dairy products from extensive systems. However, a recent study in The Netherlands identified negative associations between both organic milk consumption and increased n-3 intake and the incidence of eczema in infants.[49,50] Future research efforts should therefore focus on investigating possible impacts of consuming dairy products from less intensive dairy production systems on human health.

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Notes

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ABBREVIATIONS USED

ALA, α-linolenic acid; ANOVA, analysis of variance; BSA, bovine serum albumin; c12, lauric acid; c14, myristic acid; c16, palmitic acid; c18, stearic acid; ca, casein; cw, casein/whey protein ratio; CIS, conventional indoor system with standard milking parlor; CON, concentrate feeds; COR, conventional outdoor system with robotic milking parlor; COS, conventional outdoor system with standard milking parlor; cr, cryptoxanthin; CVD, cardiovascular diseases; DMI, dry matter intake; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acids; GRA, estimated grazing intake; GS, grass or grass/clover silage; HF, Holstein-Friesian; HPLC, high-performance liquid chromatography; HS, hay/straw; LA, linoleic acid; lu, lutein; MS, maize silage; MUFA, monounsaturated fatty acids; n-3, omega-3 fatty acids; n-6, omega-6 fatty acids; OA, oleic acid; OOS, organic outdoor system with standard milking parlor; OS, oil supplement; PUFA, polyunsaturated fatty acids; RA, rumenic acid; RDA, multivariate redundancy analysis; RM, robotic milking; SCC, somatic cell count; SFA, saturated fatty acids; tβL, β-lactoglobulin; tc, total carotenoids; uc, unknown carotenoids; VA, vaccenic acid; VT, vitamin/minerals supplement; wh, whey protein; WC, cereal whole crop; ze, zeaxanthin; αc, α-carotene; αCN, α-casein; αL, α-lactalbumin; αt, α-tocopherol; βc, β-carotene; βCN, β-casein; βLA, β-lactoglobulin A; βLB, β-lactoglobulin B; κCN, κ-casein.

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