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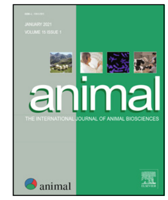

Vitamin E and beta-carotene status of dairy cows: a survey of plasma levels and supplementation practices

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ABSTRACT

Culling rate in dairy cattle has increased considerably, thereby reducing cowś longevity and raising sustainability concerns worldwide. In the last decades, feeding systems have changed towards larger inclusion of preserved forages and reduced fresh herbage, which may limit vitamin E and beta-carotene dietary supply to dairy cows. Because of higher oxidative stress, engendered by greater milk production of modern genetics, the requirement for these nutrients is increased. Therefore, this study aimed to assess the current status of vitamin E and beta-carotene of commercial dairy cows. Blood vitamin E and beta-carotene concentrations were measured in 2 467 dairy cows from 127 farms in Belgium, Germany, Iberia and The Netherlands, that were visited once. Five cows were randomly selected per lactation stage per farm: Dry (between 30 and 1 day(s) before calving), Very-early (from calving until 15 days in milk (**DIM**)), Early (between 16 and 119 DIM), and Mid-late (from 120 DIM onwards). In addition, a survey was conducted to retrieve data on vitamin E and beta-carotene supplementation and feeding practices. Vitamin E and beta-carotene blood concentrations dropped considerably around calving. Among all surveyed cows, more than 75 and 44% were deficient in vitamin E and beta-carotene (i.e., blood concentration below 3.0 and 3.5 mg/l, respectively). Of the Very-early group, more than 97 and 78% of the cows were deficient in vitamin E and beta-carotene, respectively, with respective blood concentrations of 1.15 and 2.71 mg/l, which was significantly lower than the other lactation stages. Vitamin E and beta-carotene blood concentrations, as well as their supplementation levels, significantly varied among countries. Vitamin E and beta-carotene blood concentrations were positively related to the total estimated daily intakes of vitamin E and beta-carotene. Therefore, blood concentrations of vitamin E and beta-carotene depend on their respective level of intake, which is generally below recommendations and varies greatly between countries. Supplementation could contribute to provide cows with adequate amounts of vitamin E and beta-carotene all along the lactation, to ensure their lifetime performance and improve their fertility.

Implications

High yielding dairy cows may undergo oxidative stress, which may decrease their fertility and performance. Dietary antioxidants, like vitamin E and beta-carotene, are known to reduce this drawback. We showed that vitamin E and beta-carotene blood levels varied considerably between lactation stages and between countries. Especially in Very-early lactation, the levels were often low, which may reduce the dairy cow's performance and fertility. This survey clearly demonstrates that feeding green herbage or supplementing helps improving cow's vitamin E and beta-carotene status, which may contribute to ameliorate dairy cows' fertility and performance for a more sustainable milk production worldwide.

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Introduction

Sustainability is an increasing challenge for today's dairy industry which is currently working at reducing methane and nitrogen excretion, improving animal welfare, performance, and cow longevity. The latter is closely related to herd replacement rate and acts of culling (Mohd Nor et al., 2014). Compton et al. (2017) showed in a meta-analysis that the rate of replaced dairy cows augmented by 1.4% per year since 1990. Almost 50% of culling acts were motivated by poor udder health (23%) and infertility (26%; Hemme et al., 2016). For instance, in the UK over the period 1975–82 to 1995–98, Royal et al. (2000) found that pregnancy rate to first service declined at an average rate of 1% per year. This increasing turnover endangers cow's longevity and raises sustainability concerns for the dairy industry.

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Sub-optimal fertility syndrome expressed by modern cows is seen as being multifactorial. Herd management and nutrition must be adapted to allow high milk producing cows to cope with their higher metabolic needs (Chagas et al., 2007; De Bie et al., 2016). Pryce et al. (2004) stated that greater milk production implied higher nutritional requirements, which without adaptation of the cow's diet may lead to negative energy balance and increased oxidative stress. In addition to the higher requirements, oxidative activity has been shown to exceed neutralizing capacity of antioxidants naturally produced by the organism (Celi and Gabai, 2015). In complement to these enzymatic antioxidants, antioxidants like vitamin E and beta-carotene can be supplied through the feed (Halliwell et al., 1995; Halliwell, 1996; Sies and Stahl, 1995).

Although antioxidant content of roughages varies greatly due to numerous factors (e.g., location, climate), herbage contains a lot more beta-carotene and vitamin E than preserved feedstuffs like grass and maize silages or hay (Kalač, 2012). Last decades, however, feeding systems have been changing towards larger inclusion of preserved forages and less fresh herbage (Dillon, 2018). Consequently, modern dairy cows are having limited supply of dietary antioxidants, while experiencing higher oxidative stress (Lindqvist, 2012). This mismatch may partly explain the udder health and fertility issues observed in modern cows (Leroy et al., 2008; Quintela et al., 2008).

Studies showed the relationship between vitamin E and beta-carotene status of cows with udder health or fertility. Weiss et al. (1997) found that cows with blood concentration of vitamin E below 3.0 mg/l at calving had 9.4 times more risk to present clinical mastitis during the first seven days of lactation than cows with a higher vitamin E blood concentration. In a meta-analysis, Bourne et al. (2007) and Moghimi-Kandelousi et al. (2020) showed that vitamin E supplementation of dairy cows significantly decreases the risk of retained foetal membranes postpartum. The interval from calving to first oestrus was significantly reduced by supplementing 1 000 mg of vitamin E/cow per day prior to calving and in early lactation (Campbell and Miller, 1998). Arechiga et al. (1994) and Allison and Laven (2000) showed that vitamin E supplementation had positive effects on different reproductive performance parameters (number of services per pregnancy, calving-conception interval, and pregnancy rate at 1st service after calving).

Similar effects were shown for beta-carotene. In addition to being a precursor of vitamin A, beta-carotene fed to dairy cows increases ovulation rate at the first oestrus postpartum (Kawashima et al., 2009 and 2010). Beta-carotene acts as an antioxidant for oocyte cells, while vitamin A influences follicle development and supplementation of 2 000 mg of beta-carotene per day significantly increased beta-carotene concentration in oocyte environment when fed to dry cows for 28 days, as demonstrated by Leroy et al. (2011) and De Bie et al. (2016). These authors also showed that a negative energy balance significantly reduces beta-carotene concentration in the follicle environment. Arbeiter et al. (1983) showed that a beta-carotene concentration above 3.5 mg/l of blood significantly decreased calving to oestrus interval and significantly improved ovarian functions after parturition.

The main reference for vitamin and antioxidant requirements for dairy cows is currently the one from the National Research Council (National Research Council (NRC), 2001). It is recommended to feed dry and lactating cows about 80 and 20 mg vitamin E/kg of DM intake per day, respectively, i.e., between 1 000 and 1 400 and 400 and 500 mg vitamin E/cow per day, respectively. For beta-carotene, the NRC (2001) did not indicate a specific requirement, but recommended the addition of beta-carotene and/or vitamin A in low forage diets, in periods of high immune response, and around calving. However, the Optimum Vitamin Nutrition (DSM Nutritional Products), based on Calsamiglia and Rodríguez (2012), recommends feeding dry cows between 500 and 1 000 mg beta-carotene/cow per day, and lactating cows between 300 and 500 mg/cow per day. Recommendations are usually given within large ranges as they highly depend on the forage, herbage, and concentrate amount fed, and their content in vitamin E and beta-carotene. These recommendations are not commercial practice due to costs and difficulty to assess the benefits. Last decades saw a considerable increase of milk yields and of forage types fed to cows. For these reasons, we hypothesized that cows may be at risk of low vitamin E and beta-carotene status particularly in the critical period around calving.

The development of rapid and cost-effective testing devices allows the evaluation of vitamin E and beta-carotene status on commercial dairy farms without the need for expensive High Performance Liquid Chromatography (**HPLC**) analysis. For instance, Bioanalyt GmbH (Teltow, Germany) developed several iCheck™ portable devices that allow rapid measurement of blood analytes, including vitamin E and beta-carotene (Ghaffari et al., 2019; Raila et al., 2012; Schweigert et al., 2003).

As vitamin E and beta-carotene may influence dairy cowś health and fertility, the present study aimed at assessing vitamin E and beta-carotene status of commercial Holstein cows. Although the study was performed in different European countries, it is of worldwide relevance. Their vitamin E and beta-carotene status was studied across different lactation stages, in different feeding systems, ranging from grazing to total mixed ration (**TMR**).

Material and methods

Locations and animals

Feeding practices and systems, feedstuff availability and climate are variable among countries and world regions, which may influence blood levels of vitamin E and beta-carotene of dairy cows. To get a better understanding of the antioxidant status of Holstein dairy cows, the major European breed, and the influence of husbandry and environmental conditions, we measured vitamin E and beta-carotene status of dairy cows from Belgium, Germany, Iberia (Portugal and Spain together), and The Netherlands. These countries were selected to reflect differences in climate, forage type and feeding systems. The Netherlands was chosen because of the proportion of grazing dairy farms with high milk yield. Germany was selected as TMR feeding system is commonly used there. Belgium was included because of its intermediate to Germany and The Netherlands, based on the feeding systems. Iberia was chosen because of its hotter climate, different forage bases, and a blend of non-grazing (in Spain) and grazing systems (in Portugal) (Table1).

Over two years, 2 467 cows were selected and tested once from 127 commercial dairy farms all counting a minimum of 70 high-yielding Holstein milking cows, i.e., with a milk production of over 8 500 kg/cow per year.

On each farm, 20 (minimum 15; maximum 23) cows were randomly selected according to the stratification of lactation stages and were visited once. The target was to select five cows per farm in each of the following lactation stages: Dry (between 30 days prepartum and 1 day before expected calving date), Very-early lactation (from calving until 15 DIM), Early (between 16 and 119 DIM), and Mid-late (from 120 DIM onwards). All years and countries together, the Dry group consisted of 631 cows (or heifers), the Very-early lactation group of 477, the Early group of 736, and the Mid-late group of 623 cows.

Sampling and blood analyses

From May to August 2018 and from April to July 2019, 2 467 cow blood samples were collected in 10 mL BD Vacutainer® tubes

Table 1
Description of the climate, the average temperatures and the proportion of dairy cows per feeding system (TMR, grazing, non-grazing) in the different countries selected for the survey in 2018 and 2019.

	Country							
	Belgium		Germany		Iberia		The Netherlands	
Climate[1]:	Temperate		Continental		Mediterranean		Temperate	
Item	2018	2019	2018	2019	2018	2019	2018	2019
Average temperature[2] (°C)	18.5	14.8	20.3	16.8	24.8	20.0	18.3	15.0
Number of farms	15	17	14	15	10	26	15	15
Proportion of cows fed TMR (%)	0	0	100	100	0	0	0	0
Proportion of grazing cows (%)	32	4	0	0	0	27	20	33
Proportion of non-grazing cows (%)	68	96	0	0	100	73	80	67
Proportion of cows vitamin E supplemented (%)	98	99	98	98	88	88	98	93
Proportion of cows beta-carotene supplemented (%)	10	17	15	7	0	0	56	7

TMR = Total Mixed Ration.
[1] Information retrieved from: https://www.weatheronline.co.uk.
[2] Average temperature based on the capital city of the countries from the months of sampling (May, June, July, August for 2018, and April, May, June, July for 2019). Information retrieved from: https://www.timeanddate.com/weather/.

with EDTA K2 (Becton Dickinson and Co., Franklin Lakes, USA) from the tail vein, by a local certified veterinarian. The tubes were labelled and placed immediately in a dark cooler. Within less than 8 h following sampling, blood concentration in vitamin E and beta-carotene was analysed using a portable measuring device based on photometric and fluorometric methods: iCheck™ beta-carotene and iCheck™ vitamin E (BioAnalyt GmbH, Teltow, Germany). Preservation and measurements were performed following the manufactureŕs instructions. The systems were validated by Ghaffari et al. (2019), Raila et al. (2012), Schweigert et al. (2003) and Schweigert and Immig (2007). iCheck™ results are automatically converted into mg of vitamin E or beta-carotene per litre of plasma, but results are presented as mg/l of blood, in agreement with (Raila et al., 2012) and (Ghaffari et al., 2019).

Survey of antioxidant status and feeding practices

During each farm visit, information regarding the amount of pasture, forages, concentrates, vitamin E and beta-carotene sources supplied to the cows were recorded when available. Supplement levels from the manufactureŕs declaration and ration inclusion levels were taken to calculate the daily intake.

In German farms, only TMR was fed, allowing a full analysis of the total dietary intake. This allowed a more accurate assessment of the impact of vitamin E and beta-carotene intake on the blood concentrations of these molecules. Therefore, samples of the forages and TMR for the Dry (TMR Dry) and lactating (TMR Lact) cows were collected. Analyses of the forages and TMR content of vitamin E (in form of DL-α-tocopherol acetate = 0.91 mg of DL-α-tocopherol) and beta-carotene (in form of all-trans-beta-carotene) were performed at AGROLAB LUFA GmbH (Kiel, Germany) by UV-HPLC as per the official method of the European Union (European Commission, 2009). As these analyses were only performed per farm in Germany, daily intakes of vitamin E (**DIVE**) and beta-carotene (**DIBC**) were as follows estimated per lactation stage:

$$\mathrm{DIVE\ (g/day)} = \mathrm{vitamin\ E\ content\ TMR\ Dry\ or\ Lact\ (g/kg\ DM)} * \mathrm{average\ daily\ feed\ intake\ (kg\ DM/day)}, \quad (1)$$

$$\mathrm{DIBC\ (g/day)} = \mathrm{beta-carotene\ content\ TMR\ Dry\ or\ Lact\ (g/kg\ DM)} * \mathrm{average\ daily\ feed\ intake\ (kg\ DM/day)}. \quad (2)$$

Data for the average daily feed intake were not known in these practical farms. Therefore, we assumed it to equal the estimations of NRC (2001): 12, 16, 22, and 26 kg DM per day for Dry, Very-early, Early, and Mid-late cows, respectively.

Statistical analyses

The experimental unit considered in the present study was the cow for the vitamin E and beta-carotene blood concentrations, and the farm for the estimated intake of these compounds with TMR (German farms only). To test the effect of DIM on vitamin E and beta-carotene blood concentrations, a locally estimated scatterplot smoothing function (PROC LOESS, SAS) was used. Linear regression was applied to study the relation between the average DIVE or DIBC and their respective blood levels. For all parameters studied, normality was preliminarily verified (PROC UNIVARIATE, SAS). Beta-carotene supplementation data were log-transformed to obtain a normal distribution. Blood concentration and supplementation level data were analysed by an analysis of variance (PROC MIXED, SAS). The model included the main factors of our interest (country and lactation group), as well as all their interactions, and because the study was performed over two years, the factor Year was also taken into account. Farm, within country, was included as a random factor, leading to the following model:

$$Y_{ijklm} = \mu + LG_i + Country_j + Year_k + LG * Country_{ij} + LG * Year_{ik} + Country * Year_{jk} + LG * Country * Year_{ijk} + Farm(Country)_{l(j)} + \varepsilon_{ijklm}$$

where:

- Y_{ijklm}: response variable, i.e., blood vitamin E or beta-carotene concentration;
- μ: the overall mean of the response variable;
- LG_i: Effect of the Lactation Group (i = Dry, Very-early, Early, or Mid-late);
- $Country_j$: Effect of originating country of cows (j = Belgium, Germany, Iberia, The Netherlands);
- $Year_k$: Effect of the sampling year (k = 2018, 2019);
- $LG^*Country_{ij}$, LG^*Year_{ik}, $Country^*Year_{jk}$, $LG^*Country^*Year_{ijk}$: Effects of the different interactions between the fixed factors;
- $Farm(Country)_{l(j)}$: Random effect of originating farm of cows within country (l = farm number);
- ε_{ijklm}: Residual error term.

Least Square Means (**LSMs**) were compared using the Least Significant Difference for multiple comparisons. In case of transformation, *P*-values are presented as those of the transformed data, but LSMs are shown in original dimensions. To compare the proportions of cows under recommended limits for vitamin E and beta-carotene in each lactation group, a chi-square test was applied (PROC FREQ, SAS). In all cases, differences were considered significant at $P < 0.05$.

Results

Vitamin E and beta-carotene blood concentrations according to lactation stage

Vitamin E and beta-carotene blood concentrations differed significantly between all lactation stages ($P < 0.001$; Table 2). Cows from the Very-early group showed the lowest average blood concentrations of vitamin E (1.15 mg/l) and beta-carotene (2.71 mg/l), and those in the Mid-late group, the highest (2.91 and 6.77 mg/l for vitamin E and beta-carotene, respectively). Blood concentrations were significantly lower in the Dry than in the Early group for both vitamin E (1.61 and 2.17 mg/l, $P < 0.001$) and beta-carotene (4.29 and 4.66 mg/l, $P < 0.01$), respectively.

Using a LOESS model, vitamin E and beta-carotene blood concentration evolution along the lactation was estimated for all cows and by feeding system (TMR, grazing and non-grazing) (Fig. 1). A considerable drop of vitamin E was observed around calving for all cows (Fig. 1a). The lowest vitamin E concentration (1.18 mg/l) was estimated around 5 DIM. Around 100 days after calving, the LOESS curve reached the minimum recommended vitamin E concentration (3.0 mg/l). The highest vitamin E concentration (3.19 mg/l) was estimated around 150 DIM. By feeding system, all cows reached the minimum level at around 5 days. Non-grazing cows had the lowest vitamin E level (0.98 mg/l), whereas in grazing cows, this level was 1.23 mg/l and in TMR-fed cows 1.44 mg/l. The return to minimum recommended level of 3.0 mg/l varied by feeding system, taking 71 days in non-grazing cattle, 88 days in TMR and 117 in grazing systems.

Fig. 1b shows a similar drop of beta-carotene blood concentration around calving. The lowest level (2.63 mg/l) was estimated around 5 DIM for all cows. Around 20 days after calving, the LOESS curve reached the minimum recommended beta-carotene concentration (3.5 mg/l). The highest beta-carotene blood concentration (6.97 mg/l) was estimated around 150 DIM. Feeding system had an impact on the shape of the LOESS model and on the minimum levels and time to recovery. Grazing cattle had a low beta-carotene level of 2.99 mg/l on 2 days postcalving and returned to 3.5 mg/l by 14 DIM, whereas TMR cows reached a minimum of 2.65 mg/l on 3 days after calving and returned to the recommended level by 26 DIM. Non-grazing cows had the lowest minimum level of 2.41 mg/l on 7 days postcalving and returned to recommended concentrations by 25 DIM.

Proportion of cows deficient in vitamin E and beta-carotene

Weiss et al. (1997) recommended that the blood vitamin E level should be minimally 3.0 mg/l. Considering this threshold, 75% of the 2 467 cows sampled were vitamin E deficient. As shown in Fig. 2, the proportion of deficient cows varied considerably between lactation stages ($P < 0.001$). The Very-early group showed the highest proportion of vitamin E deficient cows (97%), while these proportions were 87, 71, and 49% for the Dry, Early and Mid-late lactation groups, respectively (Fig. 2).

Of all sampled cows, 44% were deficient in beta-carotene, meaning that their blood concentration was below 3.5 mg/l, the minimum recommended by Arbeiter et al. (1983). This proportion varied also between lactation stages ($P < 0.01$), the highest being observed in the Very-early lactation stage (78%). For the Dry, Early

Table 2
Blood vitamin E and beta-carotene concentrations (mg/l) of dairy cows from the different lactation groups.

Item	Lactation group[1]					
	Dry	Very-early	Early	Mid-late	SEM	*P*-value
n	631	477	736	623		
Vitamin E	1.61[c]	1.15[d]	2.17[b]	2.91[a]	0.06	<0.001
Beta-carotene	4.29[c]	2.71[d]	4.66[b]	6.77[a]	0.15	<0.001

n = number of cows per lactation group.
[a–d]Least Square Means within a row with different superscripts differ significantly at $P < 0.05$.
[1] Dry: 30 days prior to calving until calving; Very-early: from calving until 15 DIM (days in milk); Early: between 16 and 119 DIM; Mid-late: from 120 DIM onwards.

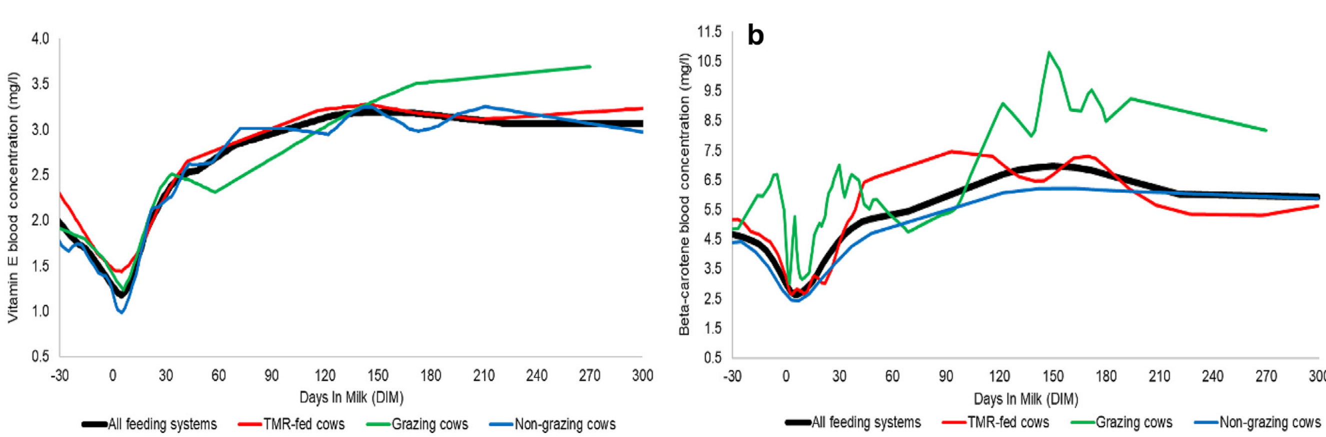

Fig 1. Observed and estimated (LOESS) mean blood concentrations (mg/l) of vitamin E (a) and beta-carotene (b) along the lactation for all dairy cows (thick black curve) and by feeding system: TMR (red), grazing (green), non-grazing (blue).

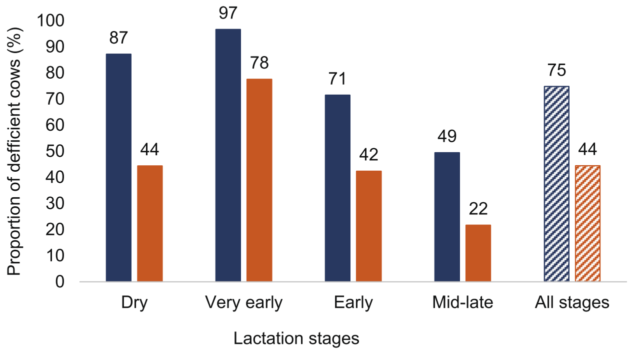

Fig 2. Proportion of dairy cows (%) deficient in vitamin E (blue bars) or beta-carotene (orange bars) per lactation stage (plain) and all stages together (hatched bars).

and Mid-late lactation groups, the proportions of deficient cows were 44, 42, and 22%, respectively (Fig. 2).

Vitamin E and beta-carotene blood levels according to country

Vitamin E and beta-carotene blood concentrations varied significantly (P < 0.001) among countries (Table 3). Cows from The Netherlands presented a higher mean concentration of vitamin E (2.37 mg/l) than those from the other countries, but not significantly higher than the values measured in the German cows (P = 0.19). The levels in these two countries were higher (P < 0.001) than those in Belgium and Iberia, which differed not significantly among them (P = 0.54). The lowest vitamin E blood concentration was found in Iberia (1.60 mg/l).

The highest mean beta-carotene blood concentrations were observed in The Netherlands (6.78 mg/l) and were significantly higher than all other countries (P < 0.001).

Beta-carotene blood concentration of cows from Iberia (2.76 mg/l) was significantly lower than for cows from all other countries (P < 0.001). Values did not differ significantly between Belgium and Germany (P = 0.19).

Vitamin E and beta-carotene blood levels according to year and interactions

A significant year effect was observed for vitamin E and beta-carotene blood concentration. Levels of both metabolites were significantly lower in 2018 compared to those of 2019 (0.94 and 2.98 mg/l for vitamin E, 4.17 and 5.04 mg/l for beta-carotene in 2018 and 2019, respectively; P < 0.001, Table 4). In addition, there appeared a significant lactation group by year interaction for vitamin E (P < 0.001) and for beta-carotene (P < 0.05) blood concentrations. For both metabolites, cows from Very-early group had the lowest values, and Mid-late cows had the highest values, in both years. However, values for each lactation group were about three times higher for vitamin E (P < 0.001), and about 20% for beta-carotene (P < 0.05), in 2019 compared to 2018. There was no significant interaction between year and country, but there was between country and lactation groups (P < 0.001, Table 4). A significant (P < 0.001) three-way interaction (country * lactation group * year) appeared for beta-carotene, but not for vitamin E blood concentrations (Table 4).

Blood levels according to the grazing system

No difference was observed between grazing and non-grazing systems on vitamin E blood concentration (1.96 mg/l for both systems). Blood beta-carotene concentrations in cows from grazing systems were significantly higher than cows from non-grazing systems (5.68 and 4.41 mg/l, respectively; P < 0.001). In addition, there was a significant interaction between country and grazing system (P < 0.01).

Blood levels according to mixed ration

The results of the vitamin E and beta-carotene content analysis in forages and the TMR from German farms are presented in Table 5.

Based on these results, DIVE and DIBC per lactation stage were estimated. The values obtained were averaged per lactation group and per farm and are presented in Fig. 3. Significant positive relations were observed between the estimated intake of both nutrients and their respective blood concentrations. The regression equations to estimate the blood concentrations were vitamin E (mg/l) = 0.15 + 1.88 * DIVE (R^2 = 0.41) and beta-carotene (mg/l) = 2.56 + 1.33 * DIBC (R^2 = 0.28) (P < 0.001 for both models).

Supplementation level per country and lactation stage

Average vitamin E supplementation differed significantly between countries and lactation stages (P < 0.05). This was, however, mainly caused by the high supplementation of Dry cows in The Netherlands, and overall a lower supplementation of cows in Iberia (Fig. 4a).

Supplementation levels of beta-carotene varied between countries and lactation stages. In Iberia, cows were not supplemented at all. In the other countries, Dry cows received higher beta-carotene supplementation compared to cows of other lactation stages (Fig. 4b).

The relation between dietary supplementation and blood concentration of vitamin E and beta-carotene could not be studied, because of the large variation in basal dietary intake and supplementation between farms. For German farms only, which were all feeding a TMR to their cows, this variation is smaller, since the vitamin E and beta-carotene levels of the TMR were measured. The effect of vitamin E supplementation on blood level could be estimated. The regression coefficient was 0.31 mg vitamin E/l blood per gram dietary vitamin E supplemented (P < 0.05). The effect of beta-carotene supplementation on the blood levels could not be well estimated, because few farms gave such supplement, and not in all lactation groups.

Table 3
Country effect on vitamin E and beta-carotene blood concentrations (mg/l) of dairy cows.

Item	Country				SEM	*P-value*
	Belgium	Germany	Iberia	The Netherlands		
n	627	574	693	573		
Vitamin E	1.68[b]	2.19[a]	1.60[b]	2.37[a]	0.10	<0.001
Beta-carotene	4.19[b]	4.69[b]	2.76[c]	6.78[a]	0.26	<0.001

n = number of cows per country.
[a–c]Least Square Means within a row with different superscripts differ significantly at P < 0.05.

Table 4
Effect of country (C), lactation group (LG), year (Y) and their interactions on vitamin E and beta-carotene blood concentrations (mg/l) of dairy cows.

Lactation group[1]	Year	Country: Belgium	Germany	Iberia	The Netherlands	*All countries*
Vitamin E blood concentration (mg/l)						
Dry	2018	0.71	1.03	0.25	1.02	*0.75*
	2019	2.18	2.85	2.17	2.69	*2.47*
	Both years	*1.45*	*1.94*	*1.21*	*1.85*	*1.61*
Very-early	2018	0.35	0.60	0.05	0.87	*0.47*
	2019	1.70	1.99	1.61	2.03	*1.83*
	Both years	*1.02*	*1.29*	*0.83*	*1.45*	*1.15*
Early	2018	0.80	1.25	0.55	1.60	*1.05*
	2019	3.11	3.45	3.09	3.47	*3.28*
	Both years	*1.96*	*2.35*	*1.82*	*2.54*	*2.17*
Mid-late	2018	1.06	1.67	0.93	2.37	*1.51*
	2019	3.54	4.66	4.14	4.94	*4.32*
	Both years	*2.30*	*3.17*	*2.54*	*3.66*	*2.91*
Overall	2018	0.73	1.14	0.44	1.47	*0.94*
	2019	2.63	3.24	2.75	3.28	*2.98*
	Both years	*1.68*	*2.19*	*1.60*	*2.37*	
Beta-carotene blood concentration (mg/l)						
Dry	2018	3.82	3.89	1.95	5.63	*3.82*
	2019	3.53	5.54	3.17	6.76	*4.75*
	Both years	*3.68*	*4.72*	*2.56*	*6.19*	*4.29*
Very-early	2018	2.13	2.18	1.57	3.84	*2.43*
	2019	2.90	0.30	2.01	3.73	*2.98*
	Both years	*2.51*	*2.74*	*1.79*	*3.78*	*2.71*
Early	2018	3.91	4.28	2.35	6.55	*4.27*
	2019	4.90	5.22	3.25	6.79	*5.04*
	Both years	*4.4*	*4.75*	*2.8*	*6.67*	*4.66*
Mid-late	2018	5.41	5.58	2.95	10.65	*6.15*
	2019	6.93	7.54	4.85	10.27	*7.40*
	Both years	*6.17*	*6.56*	*3.9*	*10.46*	*6.77*
Overall	2018	3.82	3.98	2.20	6.67	*4.17*
	2019	4.56	5.40	3.32	6.89	*5.04*
	Both years	*4.19*	*4.69*	*2.76*	*6.78*	
Number of cows						
Dry	2018	72	70	54	75	*271*
	2019	84	75	127	74	*360*
	Both years	*156*	*145*	*181*	*149*	*631*
Very-early	2018	62	56	44	61	*223*
	2019	53	68	81	52	*254*
	Both years	*115*	*124*	*125*	*113*	*477*
Early	2018	101	79	54	82	*316*
	2019	91	88	156	85	*420*
	Both years	*192*	*167*	*210*	*167*	*736*
Mid-late	2018	58	67	46	71	*242*
	2019	106	71	131	73	*381*
	Both years	*164*	*138*	*177*	*144*	*623*
Overall	2018	293	272	198	289	*1 052*
	2019	334	302	495	284	*1 415*
	Both years	*627*	*574*	*693*	*573*	*2 467*
SEM[2]	Country	Lactation group	Year			
Vitamin E	0.10	0.06	0.06			
Beta-carotene	0.26	0.15	0.16			
P-values	Country (C)	Lactation group (LG)	Year (Y)	C * LG	C * Y	LG * Y
Vitamin E	<0.001	<0.001	<0.001	<0.001	0.09	<0.001
Beta-carotene	<0.001	<0.001	<0.001	<0.001	0.13	<0.05

[1] Dry: 30 days prior to calving until calving; Very-early: from calving until 15 DIM (days in milk); Early: between 16 and 119 DIM; Mid-late: from 120 DIM onwards.
[2] SEM for both years together [slightly rounded off].

Table 5
Average and SD of vitamin E and beta-carotene contents in forages and TMR (mg/kg DM) of German dairy farms in 2018 and 2019.

Item	Vitamin E 2018	Vitamin E 2019	Beta-carotene 2018	Beta-carotene 2019
n	14	15	14	15
Grass silage	85 ± 28	87 ± 39	228 ± 49	265 ± 80
Maize silage	38 ± 33	37 ± 30	32 ± 38	33 ± 31
TMR Dry cows	65 ± 53	92 ± 41	75 ± 55	102 ± 63
TMR Lactating cows	47 ± 15	63 ± 18	56 ± 44	98 ± 26

n = number of farms per year; TMR = Total Mixed Ration.

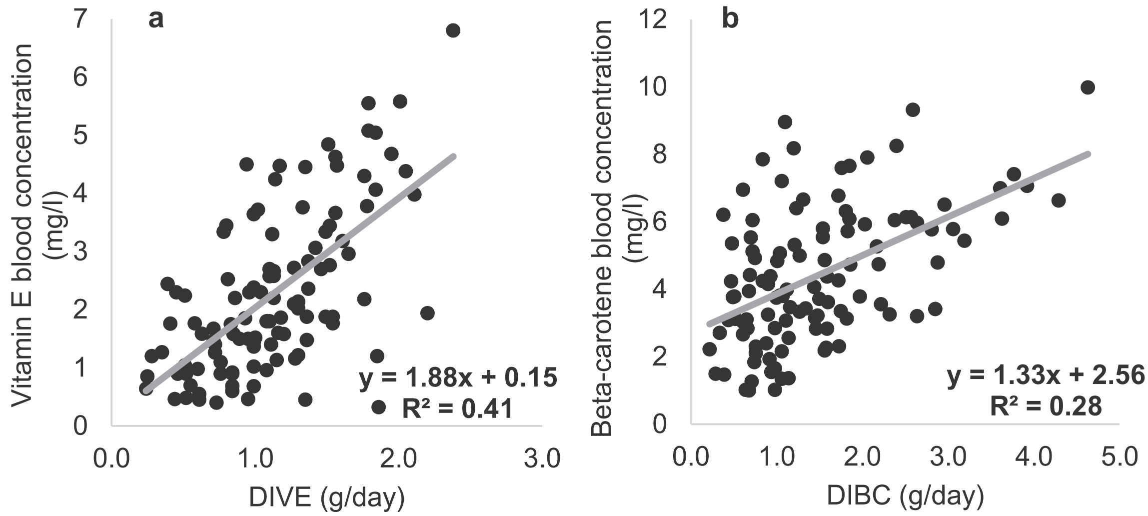

Fig 3. Blood concentrations (mg/l) versus average estimated daily intakes (g/day) of vitamin E (DIVE, a) and of beta-carotene (DIBC, b) of TMR-fed German dairy herds.

Discussion

The present study aimed to assess the status of vitamin E and beta-carotene of commercial dairy cows across Europe, which may be also relevant for numerous other parts of the world. Blood vitamin E and beta-carotene concentrations were measured in 2 467 dairy cows from 127 farms across Europe. About five cows were randomly selected per farm and per lactation stage (Dry, Very-early, Early and Mid-late). Also, the differences in vitamin E and beta-carotene blood concentrations between some countries were evaluated. These countries represent differences in climate (and, consequently, the type of forages available), and feeding practices, that could affect these blood values. Additionally, the effect of total dietary intake of cows in German TMR farms on blood concentrations was studied.

Cows in Very-early lactation, i.e., between calving and 15 DIM, presented the lowest vitamin E and beta-carotene blood concentrations compared to cows in other stages. In addition, the LOESS model revealed that vitamin E and beta-carotene follow a similar pattern along the lactation. It was noticeable that both analytes are severely depleted in cows around calving. This finding is in agreement with the observations of Weiss et al. (1997 and 2009). The present study not only confirms their conclusions but also extends their validity to cows raised in commercial farms. The low values of vitamin E and beta-carotene blood concentrations around calving may be explained by the oxidative stress engendered by the low feed intake after parturition, and the high energy demand of the dairy cow to cope with the calving process, and switching on the lactation (Ingvartsen, 2006; LeBlanc, 2010). Just after calving, cows undergo high lipomobilization, which rapidly increases blood concentration of non-esterified fatty acids (Leroy et al., 2011). This leads to excessive oxidative activity and a high use of antioxidants, like vitamin E and beta-carotene (Spears and Weiss, 2008). In addition, it was demonstrated by Weiss et al. (1997) that low vitamin E blood concentration increased the risk to retain foetal membranes after calving. This can cause a metritis, which compromises the following oestrus and pregnancy. Also, cows with low beta-carotene blood levels often present low concentration of beta-carotene and vitamin A in the follicle environment. Low vitamin A level delays follicle maturation, and low beta-carotene level decreases oocyte quality (De Bie et al., 2016). Hence, the present study showed that the 4 weeks around calving seems to be a critical period for dairy cows to maintain their vitamin E and beta-carotene blood levels to support their health and fertility.

The Early group, i.e., cows between 16 and 119 DIM, presented significantly higher blood vitamin E and beta-carotene concentrations than Very-early cows. Indeed, as it appears from the LOESS model, vitamin E and beta-carotene blood levels progressively increase after calving. This result may be due to the gradual increasing feed intake after calving, which reduces weight loss engendered by the start of milk production and, consequently, limits oxidative activity. Nevertheless, 71% of Early lactation cows of this survey are still deficient in vitamin E.

Vitamin E reached minimal recommended concentration (3.0 mg/l; Weiss et al., 1997) in cows of 100 DIM on average. This varied by feeding system: cows taking the longest time to recover were in the grazing herds (117 days), the shortest in the non-grazing herds (71 days), and 88 days in TMR-fed herds. All cows had a minimum at about day 5 postcalving. Non-grazing cows had the lowest minimum level, TMR the highest. The shapes of the LOESS curves are similar for all groups until approximately 30 DIM, probably driven by increases in feed intake. At 30 DIM, blood vitamin E concentration of the grazing cows appears to drop, or increase slower, which probably reflects grass intake being limited, as cows reach the lactation peak typically around days 50–60 (Knight, 2001). Also, the non-grazing systems had the fastest recovery, because supplementation may have typically been through concentrate feed offered in relation to milk yield. Therefore, as milk yield increased, supplementation increased. However, this was not the case, or to a lower extent, in TMR herds and in grazing herds. The difference in the shape of the curve and time to recovery has implications for cow health, particularly mastitis and fertility, and for feeding management. All cows appear to need higher levels of supplementation in the dry period to reduce the drop in vitamin E status, and also a higher level of supplementation in the first 100 days of lactation to allow a more rapid recovery of vitamin E blood concentration above the 3.0 mg/l recommended (Weiss et al., 1997).

The longer time required for reaching recommended values of vitamin E in blood, compared to beta-carotene, may be due to a relatively higher requirement of the cow for this vitamin, to a relatively lower dietary supply, or to a relatively higher recommended level. According to Meglia et al. (2004), to avoid deficiency in vitamin E around calving, dry cowś blood level must be above 5.4 mg/l. In the present study, only two cows out of 631 Dry cows reached this level. The mean vitamin E blood concentration was only 1.61 mg/l for the Dry group, from which 87% of the cows were deficient. This low value may be due to the higher energy and nutrient requirements for the last trimester of the

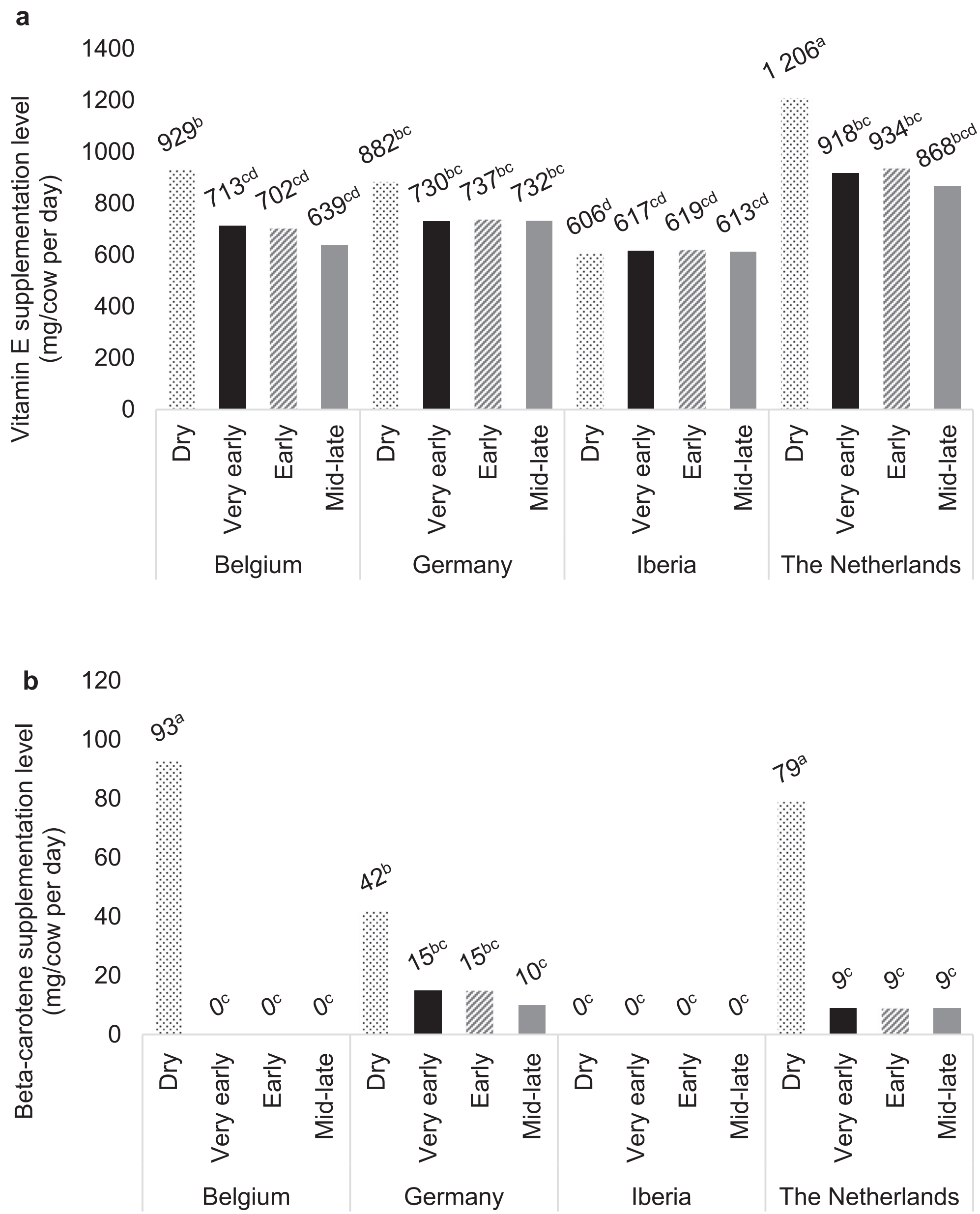

Fig 4. Average supplemented levels of vitamin E (a) and beta-carotene (b) to dairy cows (mg/cow per day) according to their lactation stage and country.

pregnancy (Beede, 1997), or to a low vitamin E intake. Dry cows are often fed low concentrate with a lot of preserved forages, which is in line with current recommendations to not over-feed energy, and to limit potassium intake. However, preserved forages are usually lower in vitamin E (Kalač, 2017), which consequently reduces its potential intake.

The minimal recommended beta-carotene blood concentration (3.5 mg/l; Arbeiter (1983) was reached by cows of 20 DIM on average. Feeding system has a large impact: grazing cows have shown a later and sharper drop in blood concentration before calving than cows in non-grazing or TMR systems, but to a minimum that is the highest of the three systems. Subsequently, they recover the fastest, reaching the recommended level (3.5 mg/l) by 14 DIM, 11–12 days faster than cows in the other systems that also showed lower minimum levels (Fig. 1b). This is to be expected as grass is a rich natural source of beta-carotene (Kalač, 2012), whereas the non-grazing and TMR systems would contain preserved forage of lower beta-carotene concentration and rely to a greater extent on supplementation for supply. The drops reflect the change in feed intake around calving and the increase in oxidative stress. This finding has practical implications for supplementation of beta-carotene by feeding system, with a higher requirement in TMR and non-grazing systems and a lower supplementary requirement in grazing cattle. Also, to prevent blood concentration below the critical 3.5 mg/l concentration in grazing cattle, supplementation can be for a shorter period. The duration of the deficiency has consequences on oocyte development and fertility (Leroy et al., 2011; De Bie et al., 2016). This is why feeding practices in Dry and Very-early lactation are critical and this is the key reason why we studied these periods. Although there is a high variability in beta-carotene blood concentration in grazing cows, we could speculate that the higher levels, compared to TMR-fed and non-grazing cows, are beneficial for fertility.

Despite all the knowledge acquired in the past decades about consequences of oxidative stress, the present survey shows that the proportion of cows presenting vitamin E and/or beta-carotene deficiency is still very high on commercial farms. Moreover, the large majority of cows were deficient in vitamin E and beta-carotene in the Dry and Very-early groups. Although the objectivity of the cut-off values for vitamin E (3.0 mg/l) and beta-carotene (3.5 mg/l) can be criticized, because they are based on few reproductive and mammary health parameters (Lotthammer et al., 1978; Weiss et al., 1997; Schweigert and Immig, 2007), the high proportion of cows found in vitamin E or beta-carotene deficiency may partly explain the high proportion of culling acts due to poor fertility (26%), as suggested by Hemme et al. (2016). In the present study, performed on commercial farms, no fertility parameters or milk production data could be evaluated. In future research, such data should be retrieved, allowing to confirm the conjectures suggested in the present study.

Differences in vitamin E and beta-carotene blood concentrations observed between European countries, lactation groups, and their interaction imply that farming systems, practices and feedstuffs influence vitamin E and beta-carotene status of the dairy cows. Milk production could also have an impact on vitamin E and beta-carotene blood concentrations. Production levels could differ between countries, but surveyed farms were selected for having a minimal production of 8 500 kg/cow per year, reducing such potential impact.

No significant differences could be noticed invitamin E blood concentration between grazing and non-grazing systems. This result was surprising, as pasture can be rich sources of vitamin E (Maxin et al., 2020) and other studies have shown an increase in vitamin E in goat milk with grazing (Delgado-Pertíñez et al., 2013). However, in this study, nearly all cows were supplemented with vitamin E and grazing was only part of the diet. Beta-carotene blood concentration was significantly greater in cows from grazing than from non-grazing systems. Pasture and herbage contain relatively high contents of beta-carotene (Kalač, 2012, 2017).

A large year effect appeared for both, vitamin E and beta-carotene. There are many ways to discuss and interpret these results. Different farms, randomly selected in different countries and in different regions within countries, were visited each year. The climate and the forages were different between years, although these were not assessed in the study. However, there are noticeable differences between grazing percentages in years. For vitamin E, grazing percentage had an impact for Iberia. In 2018, vitamin E blood concentration averaged 0.44 mg/l with zero cows grazing, and in 2019, it reached 2.75 mg/l with 28% grazing cows. Supplementation level was lower in 2019 at 536 mg/cow per day compared to 953 mg/cow per day in 2018. In Germany, with no grazing in either year, vitamin E was higher in 2019 (3.24 mg/l) compared to 2018 (1.14 mg/l). This is in agreement with supplementation levels that were 888 mg/cow per day in 2019 compared to 652 mg/cow per day in 2018. Temperatures were on average 4 °C lower in 2019 and this would have undoubtedly impacted the oxidative challenge and therefore the blood concentrations.

In general, vitamin E supplementation was more similar between years than beta-carotene levels: vitamin E was supplemented at 799 and 760 mg/cow per day in 2018 and 2019, respectively, and beta-carotene supplementation, in supplemented cows was 62 and 136 mg/cow per day in 2018 and 2019, respectively. Differences in grazing cows' proportions, supplemented cows' proportions, levels of dietary supplementation and in temperatures between 2018 and 2019, taken all together, align to explain the year effect and its interaction. For example, in Belgium in 2018, 32% of cows grazed compared to 4% in 2019. In Iberia, non-grazing cows were sampled in 2018, but because of an awareness of this, grazing herds were included in the 2019 survey, resulting in 28% of Iberian cows were in a grazing system. In addition, farms differed in their supplementation levels by year. Particularly for beta-carotene in Belgium, there was a large difference: in 2018, 10% of the cows were supplemented and received on average 173 mg/cow per day of beta-carotene, whereas in 2019, 17% of the cows were supplemented and received on average 648 mg/cow per day. This is reflected on the average blood concentration between 2018 and 2019, being 3.82 mg/l compared to 4.56 mg/l, respectively. In Iberia, with a higher proportion of cows in grazing system, in 2019, beta-carotene blood concentration was 3.32 mg/l, whereas in 2018, it was only 2.20 mg/l. This clearly translates to the lactation groups. For example, the dry cows showed a concentration of beta-carotene of 3.17 mg/l in 2019, compared to 1.95 mg/l in 2018. Temperatures were lower in 2019 compared to 2018 and this would have contributed to the higher blood levels in 2019. This resulted in a significant effect of both, year and year * lactation group interaction.

Dutch grazing farms showed higher beta-carotene blood level than Iberian grazing farms. This may be explained by the higher oxidative stress undergone by Iberian cows, due to higher temperatures and not supplementing any beta-carotene, compared to The Netherlands where temperatures are lower and 21% of the cows in grazing systems received beta-carotene supplementation. Hence, access to pasture helps to raise blood beta-carotene level in dairy cows, but dietary supplementation appears to be even more effective. On the contrary, access to pasture does not appear to improve vitamin E uptake.

The relation between supplementation and blood concentration of vitamin E and beta-carotene could not be verified, because of the large variation in basal dietary intake and supplementation between farms. This is due to the different types of forages, quality of these, and their inclusion in the ration. To acknowledge this, the relationship between the full ration intake and vitamin E and beta-carotene blood levels was studied in detail for TMR-fed cows in all surveyed German farms. This allowed a full dietary assessment of their daily intake estimated by DIVE and DIBC, based on the content of these analytes in the TMR. It has been found that levels of vitamin E and beta-carotene in the ration have an influence on blood concentrations of vitamin E and beta-carotene in dairy cows. This is in agreement with Weiss et al. (1997), who found that blood concentration of vitamin E was highly influenced by the vitamin E content of the ration. Similarly for beta-carotene, present findings agree with Kaewlamun et al. (2011) and Jin et al. (2015). Therefore, this survey not only confirms their conclusions but also extends their validity to dairy cows in commercial farms. TMR vitamin E and beta-carotene content values varied between 2018 and 2019, but it does not seem to be entirely caused by a year effect. Indeed, farms were different between both years, and each of them has their own feeding and supplementing practices. Hence, the observed variability in TMR content of vitamin E and beta-carotene was probably induced by the farm factor. Therefore, it appeared indispensable to analyse the TMR per farm to estimate the intake of vitamin E and beta-carotene. Findings showed that every 1 000 mg of vitamin E or beta-carotene added to the ration increased the respective blood concentrations by 1.88 and 1.33 mg/l. This clearly demonstrates the impact of supplementation on blood concentrations. Furthermore, to improve DIVE and DIBC accuracy in future research, it would be of interest to record individual feed intake, but this is difficult in commercial farms. Otherwise, an estimation of the individual feed intake of each feedstuff based on individual BW and/or milk production may be a practical method to clarify the relation between vitamin E or beta-carotene uptake and their concentration in blood.

Vitamin E and beta-carotene supplementation significantly varied according to country and lactation stage. Seventy four percent of the cows in the present survey received vitamin E below the recommended 1000 mg/cow per day (NRC, 2001). The recommended beta-carotene supplementation 300 mg/cow per day (Calsamiglia and Rodríguez, 2012) was not reached in 96% of the cows.

The present survey revealed that vitamin E and beta-carotene supplementation practices in commercial farms are very variable between European countries. Blood vitamin E and beta-carotene concentrations remain well under the recommended levels. These observations may also be of relevance for other parts of the world. The low blood concentrations of vitamin E in Very-early lactation largely increase the risk of mastitis indicated by Weiss et al. (1997), and reduce fertility parameters (Campbell and Miller, 1998). Higher blood beta-carotene level, at time of artificial insemination, increased pregnancy rate and reduced pregnancy loss (Madureira et al., 2020). Since a large proportion of the cows in the present study was considered deficient in vitamin E and/or beta-carotene, especially in the critical Dry, Very-early and Early lactation groups, total dietary intake of these nutrients appears to deserve more attention in practical nutrition.

Conclusion

Present study has shown that blood vitamin E and beta-carotene concentrations significantly vary by stage of lactation, by country and feeding system. Most of the cows in Dry, Very-early and Early groups were deficient in vitamin E, and needed about 100 days after calving to meet the minimum recommended level. During the Very-early lactation phase, the vast majority of cows are deficient in beta-carotene. Beta-carotene blood concentration needs about 20 days postcalving to recover and meet recommended blood levels. This survey clearly demonstrates that these levels can be elevated by an increased total dietary intake, which may include dietary supplementation. This may improve the performance, fertility, and longevity, and thus animal welfare, of dairy cows, as well as the sustainability of milk production worldwide.

Ethics committee

Not applicable.

Data and model availability statement

None of the data were deposited in an official repository. The study findings are available upon request.

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Declaration of interest

A.E.P. Mary, S.E. Richards, and A.K. Kies are employees of DSM, a producer of vitamins and carotenoids.

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