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## Revision of Vitamin E recommendations for dairy cows in organic agriculture: a review-based approach

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### ABSTRACT

Vitamin E is essential and supplementation to the diet is often needed to meet the requirements of farm animals. This is particularly relevant during long indoor periods where conserved forages must be fed, as conservation can degrade Vitamin E. However, synthetic vitamins are regarded as contentious inputs in organic agriculture. Therefore, the aim of this work was to evaluate if the standard recommendations for supplementation can be revised and adapted for organically managed dairy cows, on the basis of that the diets differ from those in conventional systems. A systematic literature review was conducted to assess the response to Vitamin E supplementation considering lactation and gestation stage and the composition of the basal diet. Most of the experiments that focused on animal health-related issues were conducted during late gestation and early lactation. In more recent studies reporting positive effects of Vitamin E supplementation on animal health and fertility, cows were fed conserved forages such as hay, haylage or maize silage, which all have low natural content of Vitamin E. In the studies reporting no or only minor positive effects of Vitamin E supplementation, cows were often fed diets based on grass or grass-clover silages, which reflects the structure of organic cattle diets. In conclusion, it was proposed that Vitamin E supplementation is not needed for mid and late lactating cows on pasture or fed a basal diet of grass-clover-silages. For dry and peripartum cows as well as for cows fed maize silage, hay or haylage, supplementation was strongly recommended.

### ARTICLE HISTORY

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### KEYWORDS

A-tocopherol; cattle; forages; organic livestock; requirements; synthetic vitamin

### Background: role and occurrence of Vitamin E regarding organic cattle diets

Vitamin E is a group of fat-soluble molecules that act as chain-breaking antioxidants in cell membranes where the primary function is to prevent oxidative damage by trapping reactive oxyradicals (Hogan et al. 1993). Vitamin E is essential for body functions such as growth, reproduction, immunity as prevention of diseases and protection of tissues (McDowell et al. 1996). Selenium and Vitamin E can to some extent compensate for each other, but not fully (Miller et al. 1993). Low levels of dietary Vitamin E may cause nutritional myodegeneration in young ruminants, and the dietary requirements necessary to prevent deficiency are estimated to be 10–40 international unit (IU) kg<sup>-1</sup> DM of the diet (Hidiroglou et al. 1992). Plasma levels of Vitamin E decline during late gestation and appear at the lowest levels at parturition. In experiments,

Vitamin E supplementation during the dry period has been shown to alleviate the decline in plasma Vitamin E content and to improve immune functions (Hogan et al. 1992; Politis et al. 1995). Vitamin E supplementation during the dry period can reduce rates and duration of infections and incidence of mastitis as well as somatic cell counts in the milk (Smith et al. 1997; Weiss et al. 1997; Politis et al. 2012); and can also reduce incidences of retained placenta (Miller et al. 1993). Furthermore, Vitamin E supplementation has been shown to improve the performance and meat quality (shelf life) of beef cattle, particularly from animals on feedlots (Liu et al. 1995; Secrist et al. 1997). Sheep adaptive immune system and meat shelf-life were also improved by Vitamin E supplementation (Tengerdy 1990; McDowell et al. 1996). Thus, adequate supply for cattle with Vitamin E is essentially important (NRC 2001, 2007; National Academies of Sciences Engineering and Medicine 2016, 2021). However, the question is, what are the adequate forms and amounts of supply in different production systems, like, in the current case, organic agriculture.

### ***Vitamin E isomers and bioavailability***

The group of molecules in the Vitamin E family are eight naturally occurring compounds, four α-, β-, γ- and δ- tocopherols and the corresponding four tocotrienols. The molecules differ in their side-chain saturation and degree of methylation of their chromanol heads. α- tocopherol is the major form found in green parts of plants, as in forages, and in higher organisms. Further, α-tocopherol is the form with the highest Vitamin E activity (Jensen and Lauridsen 2007), as it is found in significant levels in blood and in tissues (Hidiroglou et al. 1992). This because α-tocopherol is preferentially retained by the hepatic α-tocopherol transfer protein (Atkinson et al. 2019).

Out of the eight possible isomeric configurations of the α-tocopherol molecule, the RRR isomer is the only form occurring naturally in plants. Synthetic manufactured α-tocopherol, all-rac-α-tocopherol, consists of an equimolar mixture of all eight (RRR, RRS, RSS, RSR, SRR, SSR, SRS, and SSS, where the letters indicate the configuration at the three chiral centres), and the RRR-isomer comprise consequently 12.5%. The natural form, RRR-α-tocopherol, can be extracted and isolated using chemical and physical methods from vegetable oil deodoriser distillates, which are by-products from refining vegetable oils (Quek et al. 2007).

Due to price, all-rac-α-tocopherol acetate is the most sold supplement form of Vitamin E used in livestock farming. The esterified all-rac-α-tocopherol, all-rac-α-tocopheryl acetate, is defined as the international standard of Vitamin E with an activity of 1 international unit (IU) mg<sup>-1</sup>, while the bioactivity of RRR-α-tocopheryl acetate is regarded to have an activity of 1.36 IU mg<sup>-1</sup>. The endogenous bioavailability of the different isomers depends on factors like animal species and age, organs, proportion of all-rac-α-tocopheryl acetate in the diet, dosage and time (Meglia et al. 2006; Jensen and Lauridsen 2007; Lashkari et al. 2019). In dairy cattle, the RRR-isomer accounts for 84–88% of all isomers in blood and milk (Meglia et al. 2006; Slots et al. 2007; Kidane et al. 2015), irrespective of the type and quantity of Vitamin E supplementation. Thus, the bioavailability of RRR α-tocopheryl acetate relative to all-rac α-tocopheryl acetate is at least 2:1 in dairy cattle, a difference used by National Academies of Sciences Engineering and Medicine (2021). Both synthetic all-rac-α-tocopherol and extracted natural RRR-α-tocopherol are usually acetylated as the acetate ester is more stable during handling and storage than the alcohol form.

### ***Vitamin E in feed***

Considering the high bioavailability of the natural isomer RRR α-tocopherol, as discussed above, it appears important to refer to its native availability from feed components. Forages in the vegetative stage, as in pasture, have high contents of α-tocopherol, and grazing animals will in most situations get adequate amounts of Vitamin E (NRC 2001, 2007; National Academies of Sciences Engineering and Medicine 2021). However, in most parts of Europe, ruminants are to relevant extent kept

indoors and fed conserved forages. Concentration of  $\alpha$ -tocopherol varies between forage plants and conserved forages. In general, the concentration declines with plant maturity (Ballet et al. 2000), during wilting and during storage of silage and hay (Hidiroglou et al. 1976; Hakkarainen and Pehrson 1987; Ballet et al. 2000; Lindqvist et al. 2012). Ensiling causes less loss of  $\alpha$ -tocopherol than haymaking (Hidiroglou et al. 1994; Jukola et al. 1996; Shingfield et al. 2005), and grass-legume silages have generally much higher concentrations of  $\alpha$ -tocopherol than whole-crop grains and maize silages (Jensen 2003; Weiss and Wyatt 2003). The effect of the use of additives in silage making is less conclusive, but lactic acid fermentation seems to favour preservation of  $\alpha$ -tocopherol (Müller et al. 2007; Lindqvist et al. 2012). Thus, due to harvest stage, conservation and storage losses, the basal feeding of conserved and stored forage during long indoor feeding periods may not meet the requirements for Vitamin E, and supplementation is needed. Ingredients used in concentrate mixtures to ruminants, e.g. maize (*Zea mays*), small grains and oil seed meal (i.e. meal from e.g. soybean (*Glycine max*)), rape seed (*Brassica napus* subsp. *napus*), and sunflower (*Helianthus annuus*)), have in general low concentrations of  $\alpha$ -tocopherol (INRA-CIRAD-AFZ 2017–2021), and if heat treated, during processing and pelleting of concentrate mixtures, it can be lost (Anderson and Sunderland 2002).

In the NorFor feeding system (Volden 2011), used in the Scandinavian countries, the recommendations for dairy cattle are the same as in NRC (2001), which is 1.6 and 0.8 IU kg<sup>-1</sup> body weight (BW) per day in the dry and lactation period, respectively. When the NRC (2001) recommendations for dairy cattle were revised, it was taken into account that husbandry practise had changed with strong reductions in the grazing period and proportion of grazed forage in the diet, and concurrent increases in period and amount of feeding of conserved forages and proportion of cereal concentrate in the diet, which all led to reduced intake of natural sources of Vitamin E. It is important to note that the diet of dairy cows in North America usually has high proportions of maize silage, which is a poor forage source of  $\alpha$ -tocopherol. The NRC (2001) was revised again and published in 2021 (National Academies of Sciences Engineering and Medicine 2021). For dry and lactating dairy cows, the Vitamin E supplementation recommendation remained the same as in NRC (2001), but for the last 3 weeks of gestation the new recommendation was elevated (3 vs 1.6 IU kg<sup>-1</sup> BW). The INRA feeding system for ruminants (INRA 2018) recommends that dry, gestating dairy cows are supplemented 25 IU Vitamin E per kg dry matter intake (DMI). For lactating dairy cows fed a diet with less than 40% concentrate, the recommended Vitamin E supplementation is 15 IU per kg DMI, which corresponds to about 0.5 IU kg<sup>-1</sup> BW, and 40 IU kg<sup>-1</sup> DMI for lactating dairy cows with <40% concentrate in the diet.

### **Vitamin E supplementation in organic ruminant systems**

The vitamin supplementation recommendations used in organic livestock production across Europe are the same as the respective national standards, and in practice high safety margins are often added (Leiber et al. 2022; Varga et al. 2022). This may not always be adequate, since the organic feeding regimes differ from conventional, which leads to differences in the natural supply of vitamins (Witten and Aulrich 2019). While this is an issue for B vitamins in poultry (Leiber et al. 2022), it is related to lipophilic vitamins like Vitamin E in cattle (Varga et al. 2022). The relevant difference affecting Vitamin E abundance in ruminant feed is based on prescriptions of pasture access and restrictions to the use of concentrates in the organic regulations (European Parliament and the Council of the European Union 2018).

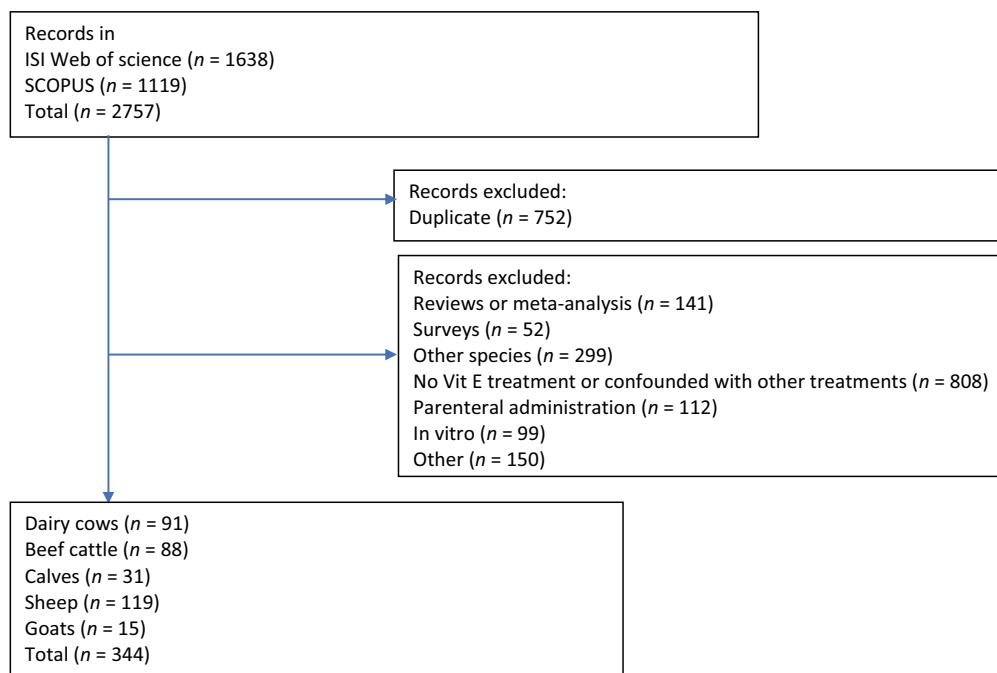
Synthetic Vitamin E is, because of the production process and the solvents involved, regarded as contentious input in organic animal production, as the animal diet should be based on naturally derived feeds (European Parliament and the Council of the European Union 2018). Synthetic vitamins may be used only if the Vitamin E requirement of the animal is not being met from the diet (European Commission 2021). In order to meet the organic principle of naturalness, contentious inputs that are still present in organic production chains, thus need to be further reduced

or completely phased out (Varga et al. 2022). A compromise could be that RRR- $\alpha$ -tocopherol extracted from vegetable oils, which are of natural origin and comply as such with the current organic standards, can be used (European Commission 2021). However, due to restrictions on the use of concentrate in the diet (<40% of the annual DM intake) and the requirement of maximum use of grazing pasturage (European Parliament and the Council of the European Union 2018), the need for Vitamin E supplementation may be lower in organic ruminant production than the current standard recommendation. Therefore, critical revision appears necessary of the relationships between basal feed type and quality, forage to concentrate ratio, stage in animal production cycle and the need for Vitamin E supplementation.

Against this background, a systematic literature review was conducted to analyse the functions of Vitamin E supplementation documented considering animal gestation and lactation stage, composition of the basal diet and relate these findings to Vitamin E, diet, and animal health status in organic production. The ultimate objective was to use this analysis as a basis for a proposed dietary Vitamin E supplementation scheme for organic ruminant production. The literature search included cattle, sheep, and goats. Here, the results for dairy cows will be presented.

### Systematic review: experimental evidence for Vitamin E effects in cattle

The focus of the study was on ruminant species common in organic dairy farming in Europe, cattle, sheep, and goats. A literature search was conducted 9 January 2020 using the Scopus and ISI Web of science databases using the following search terms ('Vitamin E' or tocopherol\* or tocopheryl\*) and (supplement\*) and (cattle\* or cow\* or calf\* or heifer\* or goat\* or sheep\* or ewe\* or lamb\*). The process of identifying relevant studies is shown in Figure 1. The inclusion criteria for selecting studies were that the studies had to, 1) be published in English language peer-reviewed journals; 2) be conducted as *in vivo* experiments with Vitamin E supplementation orally administrated as treatment to cattle, sheep or goats, 3) the Vitamin E treatments should be not confounded with



**Figure 1.** Flow diagram of identification and screening process of the literature.

other supplementations, and 4) should include information about the ingredients in the diet, i.e. forage type.

A total of 2757 studies were found (Figure 1). Many records (808) were excluded because the studies did not include Vitamin E supplementation as a treatment or Vitamin E was supplemented together with Se or other minerals and the Vitamin E effect could not be separated from other effects. Studies where Vitamin E was supplemented with injection (112) were omitted. A total of 299 records were excluded because they were on other species, e.g. poultry (66), humans (61), buffalo (52), rodents (33), pig (27), and horses (14). A total of 150 studies were omitted for other reasons, such as having no information about the basal diet, only abstract was available, manuscript written in languages other than English, Vitamin E added to animal products, animals were fed toxic basal diet (lead or arsenic). The final number of studies included was 344.

The data provided by the literature included in the review were highly heterogeneous regarding control diets, dosages, target effects, animal status, etc. Thus, there was no option to generate a meta-analysis of the data, and the way chosen was a systematic compilation into overview tables and a qualitative description in text. Based on the overall outcome of the review, a Vitamin E supplementation scheme for organic dairy production was proposed.

A total of 91 eligible references were reporting experiments on dairy cows. Some articles reported data from the same experiments, and the total number of independent studies was 80. In 40 of the 91 studies, the objectives were to test the effect of Vitamin E on animal health-related issues, 41 had focus on milk quality, 8 on reproduction and fertility-related issues and 3 on bioavailability of  $\alpha$ -tocopherol stereoisomers (Tables 1 and 2).

### **Animal health and fertility**

Most of the studies on health-related issues (33 studies) were carried out during the transition period, i.e. 3 weeks before and after calving (Table 1). Dairy cows are subjected to various metabolic changes and decreased immune status during the transition period, and plasma Vitamin E levels drop strongly during the last weeks of gestation and are at lowest level at calving before they start increasing again during the first month of lactation (Stowe et al. 1988; Weiss et al. 1990, 1992, 1994). One reason is that the concentration of plasma lipoproteins, the carriers of Vitamin E, also decreases strongly during the late stage of gestation (Herdt and Smith 1996). Feed intake declines during the same period, but the drop in plasma Vitamin E occurs even when the intake is maintained. Most studies reported increased plasma or serum content with supplementation (Table 1). Many found positive effects on immune status with supplementation during late gestation, such as improved functionality of leukocytes neutrophils and lymphocytes (Hogan et al. 1990; Politis et al. 1995, 1996, 2001; Meglia et al. 2006; Weiss et al. 2009; Dang et al. 2013; De et al. 2014), and reduced indicators of oxidative stress measured in blood (Brzezinska-Slebodzinska et al. 1994; Simpson et al. 1998; Bouwstra et al. 2009; Aggarwal et al. 2013; Aggarwal and Chandra 2018). Furthermore, supplementation of Vitamin E reduced indications of sub-clinical mastitis as indicated by milk somatic cell count (Batra et al. 1992; Politis et al. 1995, 2004; Baldi et al. 2000; Bouwstra et al. 2008; Chandra et al. 2015) and clinical mastitis. Daily dosages of about 1000 IU in the dry period reduced incidences of clinical mastitis compared to cows that received no extra Vitamin E (Smith et al. 1984; Chawla and Kaur 2004; Singh et al. 2020). In the study by Weiss et al. (1997), daily Vitamin E supplementation per cow of 1000 IU Vitamin E during the dry period, 4000 IU in the last 14 days before calving, and 2000 IU thereafter was needed to reduce incidence of mastitis compared to 1000 IU in the dry period and 500 IU thereafter. However, there are studies conducted during the peripartum period that found no or only minor differences between Vitamin E treatments with respect to animal health parameters (Batra et al. 1992; Weiss et al. 1994; Wichtel et al. 1996; Schäfers et al. 2018). A few studies conducted during lactation, i.e. after the peripartum period, reported positive effects of Vitamin E supplementation on animal health parameters (Schingoethe et al. 1979; Hogan et al. 1990; Rahmani et al. 2015), but most found no differences



**Table 1.** Effects of Vitamin E supplementation to dairy cows on plasma or serum content of tocopherol (B-T), indicators for antioxidant status or animal health in blood (B-P), milk somatic cell count (S), mastitis (M), retained placenta (R), and fertility indicators (F).

References	C <sup>1</sup>	Obj <sup>2</sup>	ED <sup>3</sup>	Phase <sup>4</sup>	VitE <sup>5</sup>	FD <sup>6</sup>	MF <sup>7</sup>	α-toc <sup>8</sup>	F/C <sup>9</sup>	Response					
										B- T <sup>13</sup>	B- P <sup>14</sup>	S <sup>15</sup>	M <sup>16</sup>	R <sup>17</sup>	F <sup>18</sup>
Aggarwal et al. (2013)	IN	H	Co	D-E	0,1000	GF+GS	85%GF	21	64/36	+	NR	NR	NR	NR	NR
Aggarwal and Chandra (2011)	IN	H	Co	D-E	0,1000	GF+H	50%GF	NR	NR	+	NR	NR	NR	NR	NR
Aggarwal and Chandra (2018)	IN	H	Co	D-E	232,2320	H/S	50%H	NR	38/62	+	NR	NR	NR	NR	NR
Al-Mabruk et al. (2004)	UK	Q	Co	M	1500,3000	GLS	100% GLS	12	60/40	+	NR	NR	NR	NR	NR
Amirfard et al. (2016)	IR	P, H	Co	D-E	0,9600	MS+LH	72% MS	NR	44/56,33/66	0	0	NR	NR	NR	NR
Atwal et al. (1991)	CA	Q	Ch	M	0,7800	MS+LS/GLH	50% MS	NR	50/50	+	NR	NR	NR	NR	NR
Atwal et al. (1990)	CA	Q	Ch	E	0,7800	MS+LS+LH	50% MS	NR	50/50	+	NR	NR	NR	NR	NR
Baldi et al. (2000)	IT	Q, R	Co	D-E	0,6600	MS+GLH	50% MS	NR	50/50	0	NR	NR	NR	NR	NR
Batra et al. (1992); Hidiroglou et al. (1992)	CA	H	Co	D-E	1000,2000	LH+GH	67% LH	NR	51/49	+	NR	-	NR	0	+
Bell et al. (2006)	CA	Q	Co	NR	0,1000/5000	HL+H+MS	50%	NR	100, 88/12	+	NR	(-)	0	0	NR
Bourne et al. (2007)	UK	H	Co	D	150,2800	WCS+LS+H	HL,70%MS	NR	60/40	NR	NR	NR	NR	NR	NR
Bouwstra et al. (2008; Dobbelaer et al. (2010))	NL	H	Co	D-E	610,1864	MS/GS	26% WCS	NR	74/26	+	NR	NR	NR	NR	NR
Bouwstra et al. (2010)	NL	H	Co*	D-E	968,2773	MS/GS	85% MS	20	54/46	0	NR	NR	NR	NR	NR
Bouwstra et al. (2009)	NL	H	Co*	D	968,2773	MS/GS	74% MS	20	54/46	0	NR	NR	NR	NR	NR
Bizerinska-Slebodzinska et al. (1994)	US	H	Co	D	0,1000	GS	75% MS	20	54/46	0	NR	NR	NR	NR	NR
Bizórska et al. (1999)	PL	Q	Ch	E/M	225,944	GS	GS	NR	63/37	NR	NR	NR	NR	NR	-
Campbell and Miller (1998)	US	R	Co	D	0,1000	GH	GH	NR	NR	+	NR	NR	0	+	NR
Chandra et al. (2018)	IN	H	Co	D-E	0,1000	H+S	50%H	NR	40/60	+	NR	NR	NR	NR	NR
Chandra et al. (2013); Chandra et al. (2014)	IN	H	Co	D-E	0,1000	H+MS	58% H	NR	34/66	+	NR	-	-	NR	NR
Chandra et al. (2015)	IN	H	Co	D-E	0,1000	GF+MA	83% GF	NR	66/33	NR	NR	-	-	NR	NR
Chandra et al. (2012)	IN	H	Co	D-E	0,1000	GF	NR	NR	NR	+	NR	NR	NR	NR	NR
Charmley and Nicholson (1994)	CA	Q	Ch	E	0,8000	WCS+LS	66% WCS	28	50/50	NR	NR	NR	NR	NR	NR
Charmley et al. (1993)	CA	Q	Co	M	0,8000	GLS	GLS	NR	51/48	+	NR	NR	NR	NR	NR
Chatterjee et al. (2005); Chatterjee et al. (2003)	IN	H	Co	D-E	0,1000	GF ad lib	NR	NR	+	+	-	-	NR	NR	NR
Chawla and Kaur (2004); Chawla et al. (2003)	IN	H/Q	Co	D-E	0,1000	GF+S	85% GF	11	64/36	+	0	NR	-	NR	NR
Dang et al. (2013)	IN	H	Co	D-E	0,1000	GF	GF	11,12	NR	NR	+NR	NR	NR	NR	NR
De et al. (2014)	IN	H/R	Co	D-E	0,2000	GF	GF	13	NR	+NR	NR	0	NR	NR	+
De Luca et al. (1957)	US	Q	Co	E	0,001%	MS + LH	MS + LH	NR	NR	+NR	NR	NR	NR	NR	NR

(Continued)

Table 1. (Continued).

References	C <sup>1</sup>	Obj <sup>2</sup>	EP <sup>3</sup>	Phase <sup>4</sup>	VitE <sup>5</sup>	FD <sup>6</sup>	MF <sup>7</sup>	α-toc <sup>8</sup>	F/C <sup>9</sup>	Response					
										B- T <sup>13</sup>	B- P <sup>14</sup>	B- S <sup>15</sup>	M <sup>16</sup>	R <sup>17</sup>	F <sup>18</sup>
Deaville et al. (2004)	UK	Q	Co	E	600/1200 0.596/429	GS LH	20 31	50/50 60/40	NR	NR	NR	NR	NR	NR	NR
Dunkley et al. (1968)	US	Q	Ch	E/M	0.475	LH	31	65/35	NR	NR	NR	NR	NR	NR	NR
Dunkley et al. (1967)	US	Q	Ch	E/M	0.5000	GS	19	87/13	NR	0	NR	NR	NR	NR	NR
Fauteux et al. (2016)	CA	Q	Ch	L	0.7500	MS+H	88%MS	85/15	+	0	NR	NR	NR	NR	NR
Ferlay et al. (2010); Govert et al. (2009)	FR	Q/H	Co	E	0.9616	MS+GS	61% MS	45/65	NR	NR	NR	NR	NR	NR	NR
Focant et al. (1998)	BE	Q	Co	NR	0.2500, 5000	MS+GS	75% MS	58/42	NR	NR	NR	NR	NR	NR	NR
Givens et al. (2003)	UK	Q	Co	NR	0.1000	LH +MS	NR	NR	NR	NR	NR	NR	NR	NR	NR
Gullikson et al. (1948)	US	R/H	Co	D	0.1000	LH	32	95/5	+	NR	-	-	0	-	0
Harrison et al. (1984), Harrison et al. (1986); Smith et al. (1984)	US	H	Co	E	0.810	MS+LH GS+H; MS+LH	50% 56% MS	NR	100; 88/12	+	NR	NR	NR	NR	NR
Hidirogloiu et al. (1997); Simpson et al. (1998)	CA	B/H	Co	D-E	0.1000	HL+H+MS	HL; 70%MS	NR	55/45 90/10; 50/50	0	0	NR	NR	NR	NR
Hogan et al. (1990)	US	H	Co	D-E	0.1050	MS+LH GS+H; MS+LH	60%MS; 63%GS;	NR	NR	+ 0	NR	NR	NR	NR	NR
Höjer et al. (2012)	NO	Q	Ch	M	0.2176	GLS	31	76/24	NR	NR	NR	NR	NR	NR	NR
Jackson et al. (1997)	US	H	Co*	D-L	0.1000, 2000 0.600	H GLS	NR 13:50	64/36 50/50; 60/40	NR 0	NR	0	NR	NR	NR	NR
Johansson et al. (2014)	SE	H	Co	L	0.10000	MS+GS+H	48% MS	23	73/27	NR	NR	0	(-)	NR	NR
NZ	Q	Co	E	E	0.2850	LH+MS	63% H	NR	43/57	NR	NR	NR	NR	NR	NR
Kay et al. (2005)	IR	P/Q	Ch	M	0.1900	GLS ad lib	35	84/16	+	0	NR	NR	NR	NR	NR
Khodamoradi et al. (2013)	NO	H/B	Ch	M	0.1000	GH+MS	NR	NR	NR	NR	NR	NR	NR	NR	NR
Krukovsky and Loosli (1952)	US	P/Q	Ch	D-L	0.2400	GLS, P	GLS ad lib	47/41/42	87/13; 51/49; (+); NR	0	0	NR	0	0	
Lindqvist et al. (2011)	SE	H/Q/R	Co	D-L	500,2400	GLS+GH+P	GLS ad lib	20/20:20	44/45/35 60/40	+0 (+); NR	0	0	NR	0	0
H/Q/R	Co	D-L													
Liu et al. (2008)	CN	Q	Co	E-M	0,5000, 10000	MS+LH MS+LH	66%MS 50% MS	NR	50/50	+0	NR	NR	NR	NR	NR
Lundin and Palmquist (1983)	US	Q	Co	E	0,1000	P/GS	P ad lib	30	60/40 85/1587/13	+	0	NR	NR	NR	NR
McKay et al. (2019)	IE	P	Co	L	1050,3150	GS/MS	60% GS	34	46/54	NR	NR	NR	NR	NR	NR
DK	B	Co	D-E	Co	0,1000	MS/LS	73%MS	NR	55/45	+	NR	NR	NR	NR	NR
US	Q	Ch	M-L	M	0,2000	MS or LS	LS	9	NR	+	NR	NR	NR	NR	NR
Morales et al. (2000)	CA	Q	Co	M	0,7000	MS+GS+H+S	50% MS	NR	16	NR	+	0	NR	NR	NR
Nicholson et al. (1991)	CA	Q	Co	NR	0,3000	MS+H; MS	51%MS;	NR	60/40	+	-	NR	NR	NR	NR
Nicholson et al. (1991)	US	Q	Co	D-E	0,10000	+LH	62%MS	NR	82/40; 62/38	+	-	NR	NR	NR	NR
O'Donnell-Megato et al. (2012)	GR	H	Co	D-E	300, 3000/1000	MS+HL+H	50%HL; 70%MS	12	100; 88/12	+	-	NR	NR	NR	NR
Politis et al. (2004)	CA	H	Co	D-E	0,3000										
Politis et al. (1995)															

(Continued)



Table 1. (Continued).

References	C <sup>1</sup>	Obj <sup>2</sup>	EP <sup>3</sup>	Phase <sup>4</sup>	VitE <sup>5</sup>	FD <sup>6</sup>	MF <sup>7</sup>	α-toc <sup>8</sup>	F/C <sup>9</sup>	70/30/42/58	+ +	B- T <sup>13</sup>	B- P <sup>14</sup>	S <sup>15</sup>	M <sup>16</sup>	R <sup>17</sup>	F <sup>18</sup>	Response
Politis et al. (2001)	US	H	Co	D-E	0,3000	MS+LH	77% MS; 52% LH	NR	70/30/42/59	+ +	NR	NR	NR	NR	NR	NR	NR	
Politis et al. (1996)	US	H	Co	D-E	0,3000	MS+LH	77% MS; 47% MS	NR	70/30/42/59	+ +	NR	NR	NR	NR	NR	NR	NR	
Pottier et al. (2006)	BE	Q	Ch	NR	0,12000	MS	MS	17	50/50	NR	NR	NR	NR	NR	NR	NR	NR	NR
Rahmanian et al. (2015)	IR	P/H	Co	E	0,4400	LH+MS	54% LH P	NR	38/62	NR	+ +	NR	NR	NR	NR	NR	NR	NR
Ramírez-Mella et al. (2013)	MX	Q	Ch	M	0,40000;8000,12000	P	100%MS	NR	70/30	NR	NR	NR	NR	NR	NR	NR	NR	NR
Santos et al. (2019)	BR	H	Ch	E	0,5625	MS+GLH	80%MS	18	60/40	NR	NR	0	NR	NR	NR	NR	NR	NR
Schinggaethe et al. (1979)	US	Q	Co	M	0,408	MS+GS	50%MS	NR	50/50	NR	NR	0	NR	NR	NR	NR	NR	NR
Schäfers et al. (2018)	DE	Q	Co	D-E	400;2450	MS+GS	50%MS	NR	40/60;50;50	NR	NR	0	NR	NR	NR	NR	NR	NR
Schäfers et al. (2017); Schäfers et al. (2018)	DE	H	Co	D-E	0,2327	MS+GS	GF+H	NR	NR	NR	NR	-	-	NR	NR	NR	NR	NR
Singh et al. (2020)	IN	P/Q	Co	D-E	0,1000	MS+WCS+GS	43%MS	NR	83/17	NR	NR	NR	NR	NR	NR	NR	NR	NR
Slots et al. (2007)	DE	Q	Co	M	0,2600	GS+S	80%GS	NR	77/23	NR	NR	NR	NR	NR	NR	NR	NR	NR
Stlaurent et al. (1990)	CA	Q/H	Co	E-M	0,3400	LS	LS	NR	NR	+ +	NR	NR	NR	NR	NR	NR	NR	NR
Stowe et al. (1988)	UK	H/R	Co*	E-M	0,700;3000	GLH, HL	NR	NR	NR	+ +	NR	NR	NR	NR	NR	NR	NR	NR
SE	H/R	Co*	D-L	0,500	0,700;3000	HL+MS+LH	NR	NR	NR	+ +	NR	NR	NR	NR	NR	NR	NR	NR
WE	Q/H	Co	D-E	0,2400	0,800/700	GS+LH	GS	NR	NR	NR	NR	0	NR	NR	NR	NR	NR	NR
Weiss et al. (1994)	US	H	Co	D-E	0,1000	GS+GH; MS	53% GLS;	NR	93/7;56/44	+ +	0	NR	NR	NR	NR	NR	NR	NR
Weiss et al. (1997)	US	H	Co	D-E	100/100;1000/500, 1000/4000/2000	+LH	56% MS;	23;19	88/12;50/50	+ +	0	NR	NR	NR	NR	NR	NR	NR
Weiss et al. (2009)	US	B	Co	D-E	0,2500	MS+GS+GH;	55% MS	NR	80/20;53/47	+ +	NR	-	NR	NR	NR	NR	NR	NR
Weiss and Wyatt (2003)	US	Q/H	Co	M	607; 2779;5828	MS+LH+LH	72%MS	36	53/47	+ +	NR	NR	NR	NR	NR	NR	NR	NR
Whiting et al. (1949)	US	P/Q	Ch	E	0,1000	GLH+MS	GLH ad lib	NR	66/34; 55/45	+ +	NR	NR	NR	NR	NR	NR	NR	NR
Wichiel et al. (1996)	NZ	R	Co	D-E	0,3600	GH; P	20	100	55/45	+ +	0	NR	NR	0	0	NR	NR	NR
Zened et al. (2012)	FR	Q	Ch	M	0,15100	MS	MS	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

<sup>1</sup>C = country and country codes.<sup>2</sup>Obj = Objectives of the work where H = animal health related issues; Q = milk quality, including gross composition, flavour, lipid oxidative stability, fatty acid composition and vitamin E content; P = production, R = reproductive parameters, B = Vitamin E bioavailability.<sup>3</sup>ED = experimental design, where Ch = change over, an Co = continuous, \* field experiment within herds or with herds.<sup>4</sup>Phase = production phase, where D = dry period/late gestation, E = early lactation <120DIM, M = mid lactation 121–240DIM, L = late lactation >240DIM, D-E = from dry period and early lactation, E-L = from dry period to late lactation.<sup>5</sup>VitE = Vitamin E treatment dosages in IU d<sup>-1</sup>, Treatments are separated by ". Changes in supplementation dosage within a treatment during experiment is indicated by '/'.<sup>6</sup>FD = Forages in the diet, where GF = green fodder unspecified, GH = grass hay, GLH = grass silage, GS = grass-legume silage, H = hay, HL = grass haylage, LH = lucerne hay, MS = maize silage, LS = lucerne silage, P = pasture, WCS = whole crop silage, " separate feeding before and after parturition.



<sup>7</sup>MF = DM proportion of the main forage of total forage DM, “,” separate feeding before and after parturition. NR = not reported.  
<sup>8</sup>α-toc = concentration of α-tocopherol, as mg kg<sup>-1</sup> DM, in the control diet. If the concentration were reported as IU vitamin E, concentration of α-tocopherol was calculated as vitamin E IU/1.49. NR = not reported or possible to calculate due to no information about feed intake.

<sup>9</sup>F/C = forage concentrate ratio in the diet, “,” separate feeding before and after parturition. NR = not reported.

<sup>13</sup>B-T = blood plasma or serum tocopherol content, where -, 0, + indicate reducing, no or increasing effect of VitE supplementation, respectively. NR = not reported/relevant.

<sup>14</sup>B-P = other blood parameter indicators for antioxidative status or animal health (immune response), where -, 0, + indicate negative, no or positive effect of VitE supplementation, respectively. NR = not reported/relevant.

<sup>15</sup>S = milk somatic cell count, where -, 0, + indicate reducing, no or increasing effect of VitE supplementation, respectively. NR = not reported/relevant.

<sup>16</sup>M = mastitis, where -, 0, + indicate reducing, no or increasing effect of VitE supplementation, respectively. NR = not reported/relevant.

<sup>17</sup>R = retained placenta, where -, 0, + indicate reducing, no or increasing effect of VitE supplementation, respectively. NR = not reported/relevant.

<sup>18</sup>F = fertility indicators, where -, 0, + reduced, no or improved effect of VitE supplementation, respectively. NR = not reported/relevant.

**Table 2.** Effects of Vitamin E supplementation to dairy cows on milk yield (M-Y), milk content of tocopherol (M-T), and milk quality (M-Q).

References	C <sup>1</sup>	Obj <sup>2</sup>	ED <sup>3</sup>	Phase <sup>4</sup>	VitE <sup>5</sup>	FD <sup>6</sup>	MF <sup>7</sup>	α-toc <sup>8</sup>	F/C <sup>9</sup>	M-Y <sup>10</sup>	M-T <sup>11</sup>	M-Q <sup>12</sup>	Response	
Agarwal et al. (2013)	IN	H	CO	D-E	0,1000	GF+GS	21	64/36	+	NR	NR			
Agarwal and Chandra (2011)	IN	H	CO	D-E	0,1000	GF+H	NR	NR	+	NR	NR			
Agarwal and Chandra (2018)	IN	H	CO	D-E	0,1000	H/S	50%H	38/62	0	NR	NR			
Al-Mabrik et al. (2004)	UK	Q	CO	D-E	232, 2320	GLS	100%GLS	12	60/40	0	+O			
Amirifard et al. (2016)	IR	P, H	CO	D-E	1500, 3000	MS+LH	72%MS	NR	44/56/33/66	0	NR			
Atwal et al. (1991)	CA	Q	Ch	M	0,9600	MS+LS/GLH	50% MS	NR	50/50	0	+O			
Atwal et al. (1990)	CA	Q	Ch	E	0,7800	MS+LS+LH	50% MS	NR	50/50	0	+NR			
Baldi et al. (2000)	Q	CO	D-E		0,7770	MS+LS+LH	50% MS	NR	50/50	0	+NR			
Batra et al. (1992); Hidiroglou et al. (1992)	Π	Q, R	CO	D-E	0,6000	MS+GLH	50% MS	NR	50/50	0	+NR			
Bell et al. (2006)	CA	Q	CO	D-E	1000, 2000	LH+GH	67% LH	NR	51/49	0	NR			
Bourne et al. (2007)	UK	H	CO	D-E	0,1000/500	HL+H+MS	50% HL; 70%	MS	100; 38/12	NR	+	NR		
Bouwstra et al. (2008); Dobbelaar et al. (2010)	NL	H	CO	D-E	150, 2800	WCS+LS+H	26% WCS	NR	60/40	0	+F			
Bouwstra et al. (2010)	NL	H	CO*	D-E	610, 1864	MS+GS	85% MS	20	74/26	0	NR			
Brzezinska-Slebodzinska et al. (1994)	US	H	CO	D	968, 2773	MS+GS	74% MS	20	54/46	0	NR			
Brzózka et al. (1999)	PL	Q	Ch	E/M	968, 2773	MS+GS	75% MS	20	54/46	0	NR	(+)		
Campbell and Miller (1998)	US	R	CO	D	0,30000	GS+MS	GS ad lib	NR	NR	NR	NR			
Chandra et al. (2018)	IN	H	CO	D-E	135, 3000	GS+MS	GS ad lib	NR	NR	NR	NR			
Chandra et al. (2013); Chandra et al. (2014)	IN	H	CO	D-E	0,1000	GS	GS	NR	NR	NR	NR			
Chandra et al. (2015)	IN	H	CO	D-E	0,1000	GH	GH	NR	NR	NR	NR			
Chandra et al. (2012)	IN	H	CO	D-E	0,1000	GS	GS	NR	63/37	0	NR			
Charmley and Nicholson (1994)	CA	Q	Ch	E	0,1000	GH	GH	NR	NR	0	NR			
Charmley et al. (1993)	CA	Q	CO	M	0,8000	H/S	50% H	NR	40/60	NR	NR			
Chatterjee et al. (2005); Chatterjee et al. (2003)	IN	H	CO	D-E	0,8000	MS+LH	58% H	NR	34/66	+	NR			
Chawla and Kaur (2004); Chawla et al. (2003)	IN	H/Q	CO	D-E	0,1000	GF+MS	83% GF	NR	66/33	+	0			
Dang et al. (2013)	IN	H	CO	D-E	0,1000	GF+MA	NR	NR	NR	NR	NR			
De et al. (2014)	IN	H/R	CO	D-E	0,1000	GF	66% WCS	28	50/50	0	+0	0		
De Luca et al. (1957)	US	Q	CO	E	0,8000	GLS	GLS	NR	51/48	0	+0	G		
Deaville et al. (2004)	UK	Q	CO	E	0,1000	GF	GF ad lib	NR	NR	+	NR			
Dunkley et al. (1968)	US	Q	Ch	E/M	0,1000	GF+S	85% GF	11	64/36	+	NR	+O		
Dunkley et al. (1967)	US	Q	Ch	L	0,1000	GF	GF	11;12	NR	NR	NR			
Fautoux et al. (2016)	CA	Q	Ch	L	0,2000	GF	GF	13	NR	0	NR	0		
Ferlay et al. (2010); Gobert et al. (2009)	FR	Q/H	CO	E	0,001%	MS + LH	MS + LH	NR	50/50	0	+	G,+O		
Focant et al. (1998)	BE	Q	CO	NR	600, 1200	GS	GS	20	60/40	0	+	G		
					0,596, 429	LH	LH	31	65/35	0	+	O		
					0,475	LH	LH	31	87/13	0	+	G		
					0,5000	GS	GS	19	85/15	0	NR	0		
					0,7500	MS+H	88% MS	NR	85/15	0	NR	0		
					0,9616	MS + GS	61% MS	11	45/65	0	+	+F		

(Continued)

Table 2. (Continued).

References	C <sup>1</sup>	Obj <sup>2</sup>	ED <sup>3</sup>	Phase <sup>4</sup>	VitE <sup>5</sup>	FD <sup>6</sup>	MF <sup>7</sup>	α-toC <sup>8</sup>	F/C <sup>9</sup>	M-y <sup>10</sup>	M-T <sup>11</sup>	M-Q <sup>12</sup>	Response	
Givens et al. (2003)	UK	Q	Co	NR	0, 25000, 50000	M-S+GS	75% MS	NR	58/42	0	+	0 G, 0 F		
Gulliksson et al. (1948)	US	Q	Co	NR	0, 10000	LH +MS	NR	NR	NR	0	NR	NR		
Harrison et al. (1984); Harrison et al. (1984). Harrison et al. (1986); Smith et al. (1984)	US	R/H	Co	D	0, 10000	HL	NR	95/5	NR	NR	NR	NR		
Hidiroglou et al. (1997); Simpson et al. (1998)	CA	B/H	Co	D-E	0, 10000	HL+H+MS	50% HL;	NR	100; 88/12	NR	NR	NR		
Hogan et al. (1990)	US	H	Co	E	0, 810	MS+LH	70% MS	NR	55/45	NR	NR	NR		
Hogan et al. (1992); Weiss et al. (1992)	US	H	Co	D-E	0, 1050	GS+H; MS+LH	56% MS	NR	90/10; 50/50	NR	NR	NR		
Höjer et al. (2012)	NO	Q	Ch	M	0, 2176	GLS	63% GS; 60%	MS	31	76/24	0	+	NR	
Jackson et al. (1997)	US	H	Co*	D-L	0, 10000, 2000	H	NR	NR	64/36	0	NR	NR		
Johansson et al. (2014)	SE	H	Co	L	0, 600	GLS	13;50	50/50; 60/40	0	0	0 G			
NZ	Q	Co	L	P/Q	0, 10000	MS+GS+H	48% MS	23	73/27	0	+ F			
IR	P/Q	Ch	E	P/Q	0, 2850	LH+MS	63% H	NR	43/57	0	NR	0 G		
Khadamoradi et al. (2013)	NO	H/B	Ch	M	0, 1900	GLS	GLS ad lib	35	84/16	0	NR	0 G		
Kidane et al. (2015)	US	P/Q	Ch	M	0, 1000	GH+MS	NR	NR	0	+ G+O				
Krukovsky and Loosli (1952)	SE	H/Q/R	Co	D-L	0, 2400	GLS, P	GLS ad lib	47;41;42	87/13; 51/49;	0	0	0 G		
Lindqvist et al. (2011)	H/Q/R	Co	D-L		500, 2400	GLS+GH+P	GLS ad lib	50/40	60/40	-	-	+ F		
Liu et al. (2008)	CN	Q	Co	E-M	0, 50000, 10000	MS+LH	66% MS	NR	84/16/56/	44;65;35				
Lundin and Palmquist (1983)	US	Q	Co	E	0, 1000	MS+LH	50% MS	NR	50/50	0	+ F			
McKay et al. (2019)	IE	P	Co	L	1050, 3150	P/GS	P ad lib	30	85/15;87/13	0	NR	0 G, 0 O		
DK	B	Co	D-E	M-L	0, 1000	GS+MS	60% GS	34	46/54	0	0	0 G		
US	Q	Ch	M-L	M	0, 2000	MS/LS	73% MS	NR	55/45	NR	+ NR			
CA	Q	Co	M		0, 7000	MS or LS	MS or LS	9	NR	NR	+ NR	0 G,		
Nicholson et al. (1991)	CA	Q	Co	E	0, 3000	LS	LS	16	NR	0	+ G,	+ O		
O'Donnell-Megaro et al. (2012)	US	Q	Co	NR	0, 10000	MS+GS+H+S	50% MS	NR	60/40	0/+	+ G,-P			
Politis et al. (2004)	GR	H	Co	D-E	300, 3000/1000	MS+H; MS+LH	51% MS; 62%	MS	82/40; 62/38	NR	NR	NR		
Politis et al. (2001)	US	H	Co	D-E	0, 3000	MS+LH	77% MS;	NR	70/30;42/58	NR	NR	NR		
Politis et al. (1996)	US	H	Co	D-E	0, 3000	MS+LH	52% LH	MS	77% MS; 47%	NR	NR	NR		
Pottier et al. (2006)	BE	Q	Ch	NR	0, 12000	MS	MS	17	50/50	0	+ F			
IR	P/H	Co	E	P/H	0, 4400	LH+MS	54% LH	NR	38/62	0	NR	NR		
MX	Q	Ch	M	BR	0, 4000; 8000, 12000	P	P	NR	70/30	0	NR	0 G,		
Santos et al. (2019)	BR	H	Ch	E	0, 5625	MS	100% MS	MS	60/40	0	NR	0 G, 0 O		

(Continued)

**Table 2.** (Continued).

References	C <sup>1</sup>	Obj <sup>2</sup>	ED <sup>3</sup>	Phase <sup>4</sup>	VitE <sup>5</sup>	FD <sup>6</sup>	MF <sup>7</sup>	α-toc <sup>8</sup>	F/C <sup>9</sup>	M-Y <sup>10</sup>	M-T <sup>11</sup>	M-Q <sup>12</sup>	Response	
													M-	M-
Schäfers et al. (2018)	DE	Q	Co	D-E	400, 2450	MS+GS	50%MS	NR	50/50	0	NR	+F		
Schäfers et al. (2017); Schäfers et al. (2018)	DE	H	Co	D-E	0,2327	MS+GS	50% MS	NR	40/60/50/50	0	NR	+F		
Schingoethe et al. (1979)	US	Q	Co	M	0,408	MS+GLH	80%MS	18	70/30	0	+	F,+P,-O		
Singh et al. (2020)	IN	P/Q	Co	D-E	0,1000	GF+H	NR	NR	NR	NR	+	NR		
Slots et al. (2007)	DE	Q	Co	M	0,2600	MS+WCs+GS	43%MS	NR	83/17	NR	+	NR		
Stlaurent et al. (1990)	CA	Q/H	Co	E-M	0,3400	GS+S	80%GS	LS	NR	NR	+	NR		
Stowe et al. (1988)	UK	H/R	Co	D-L	0,700, 3000	LS	NR	NR	NR	NR	+	O		
Waller et al. (2007)	SE	H/R	Co*	D-E	0,500	GLH, HL	NR	NR	NR	NR	NR	NR		
Weiss et al. (1990)	US	Q/H	Co	D-E	0,2400	HL+MS+LH	GS	NR	NR	0	+	NR		
Weiss et al. (1994)	US	H	Co	D-E	0,800, 0,700	GS+GS; MS	53%GS;	NR	93/7; 56/44	0	+	0 G		
Weiss et al. (1997)	US	H	Co	D-E	0,1000	+LH	56%MS	23/19	88/1250/50	NR	+	NR		
Weiss et al. (2009)	US	B	Co	D-E	100/100, 1000/500, 1000 +	GS+GH; MS+LH	63% GS; 60%	MS						
Weiss and Wyatt (2003)	US	Q/H	Co	M	4000/2000	GS+GH; MS	69%GS; 64%	MS	81/40	80/20/53/47	0	+	0 G	
Whiting et al. (1949)	US	P/Q	Ch	E	0,2500	+LH	35%MS; 55%	MS	22/14	66/34/5/45	0	+	0 G	
Wichtel et al. (1996)	NZ	R	Co	D-E	607, 2779, 5828	MS+LH+ LS	72%MS	MS	36	53/47	0	+	0 G	
Zened et al. (2012)	FR	Q	Ch	M	0,1000, 0,3600, 0,15100	GLH+MS	GLH ad lib	GH,P	20	100	NR	NR	NR	
						MS	MS	MS	55/45	0	NR	NR	0 G	

<sup>1</sup>C = country and country codes.<sup>2</sup>Obj = Objectives of the work where H = animal health related issues; Q = milk quality, including gross composition, flavour, lipid oxidative stability, fatty acid composition and Vitamin E content; P = production, R = reproduction parameters, B = Vitamin E bioavailability.<sup>3</sup>ED = experimental design, where Ch = change over, an Co = continuous, \* field experiment within herds or with herds.<sup>4</sup>Phase = production phase, where D = dry period/late gestation, E = early lactation <120DIM, M = mid lactation 121–240DIM, L = late lactation >240DIM, D-E = from dry period and early lactation E-L = from dry period to late lactation.<sup>5</sup>VitE = Vitamin E treatment dosages in IU d<sup>-1</sup>. Treatments are separated by ". Changes in supplementation dosage within a treatment during experiment is indicated by '/'.<sup>6</sup>FD = Forages in the diet, where GF = green fodder unspecified, GH = grass hay, GLH = grass silage, GS = grass-legume silage, GLS = grass silage, GLS = grass-legume silage, H = hay, HL = lucerne hay, MS = maize silage, LS = lucerne silage, P = pasture, WCS = whole crop silage, " separate feeding before and after parturition.<sup>7</sup>MF = DM proportion of the main forage of total forage DM, " separate feeding before and after parturition. NR = not reported.<sup>8</sup>α-toc = concentration of α-tocopherol, as mg kg<sup>-1</sup> DM, in the diet. If the concentration were reported as IU vitamin E, concentration of α-tocopherol was calculated as vitamin E IU/1.49. NR = not reported or possible to calculate due to no information about feed intake.<sup>9</sup>F/C = forage concentrate ratio in the diet, " separate feeding before and after parturition. NR = not reported.<sup>10</sup>M-Y = forage yield, where -, 0, + indicate reducing, no or increasing effect of VitE supplementation, respectively. NR = not reported/relevant.<sup>11</sup>M-T = milk tocopherol content, where -, 0, + indicate reducing, no or increasing effect of VitE supplementation, respectively, on G = gross composition, F = fat content, O = flavour score or oxidative stability. NR = not reported/relevant.<sup>12</sup>M-Q = milk quality parameters, where -, 0, + indicate reducing, no or increasing effect of VitE supplementation, respectively, on G = gross composition, F = fat content, O = flavour score or oxidative stability. NR = not reported/relevant.

between Vitamin E treatments (Lundin and Palmquist 1983; Nicholson et al. 1991; Jackson et al. 1997; Gobert et al. 2009; Kidane et al. 2015).

Based on the results of Weiss et al. (1997), NRC (2001) state that plasma  $\alpha$ -tocopherol concentrations at calving should be at least  $3 \mu\text{g ml}^{-1}$   $\alpha$ -tocopherol. To achieve this blood level, NRC (2001) recommends that dry cows and heifers need to be supplemented with  $1.6 \text{ IU kg}^{-1}$  BW, or about  $80 \text{ IU kg}^{-1}$  total DMI, of Vitamin E daily during the dry period. The updated version recommends elevating the supplementation from 1.6 to  $3.0 \text{ IU kg}^{-1}$  BW in the last 3 weeks of gestation (National Academies of Sciences Engineering and Medicine 2021), which is based on the findings by Baldi et al. (2000). In the studies that the NRC (2001) and the National Academies of Sciences Engineering and Medicine (2021) Vitamin E recommendations are based on (e.g. Hogan et al. 1990; Politis et al. 1995, 1996; Weiss et al. 1997; Baldi et al. 2000), as well as in many of the other studies listed in Table 1 reporting positive effects on immune status and udder health, cows were fed conserved forages such as hay, haylage or maize silage, which all have a low natural content of  $\alpha$ -tocopherol. In early lactation, maize silage and grain-based concentrate accounted for more than 70% of the dietary DM in the studies by Hogan et al. (1990), Politis et al. (1995, 1996), Weiss et al. (1997), and Baldi et al. (2000). More recent long-term experiments or large field experiments conducted in Europe, where grass or grass-legume silages were the main forage, showed no or only minor effects of Vitamin E supplementation on udder health (Waller et al. 2007; Lindqvist et al. 2011; Johansson et al. 2014) and even a negative effect (Bouwstra et al. 2010). In the latter study, cows with high plasma level of  $\alpha$ -tocopherol at dry off ( $>6.2 \mu\text{g ml}^{-1}$ ) had greater risk for getting clinical mastitis.

Vitamin E supplementation during the transition period reduced the incidences of retained placenta in two studies (Harrison et al. 1984; Brzezinska-Slebodzinska et al. 1994), but more studies observed no differences (Stowe et al. 1988; Batra et al. 1992; Campbell and Miller 1998; Baldi et al. 2000; Waller et al. 2007). A meta-analysis by Bourne et al. (2007) showed a significantly reduced risk for retained placenta in cows supplemented (mostly by injection) with Vitamin E in the dry period. Some studies report improved fertility or reproductive performance (Campbell and Miller 1998; Baldi et al. 2000; De et al. 2014), while others found no difference (Stowe et al. 1988; Wichtel et al. 1996; Waller et al. 2007; Lindqvist et al. 2011).

### **Milk production and quality**

Increased milk production performance with Vitamin E supplementation was found in most of the experiments conducted in India (Chawla and Kaur 2004; Chatterjee et al. 2005; Aggarwal and Chandra 2011; Aggarwal et al. 2013; Chandra et al. 2015; Singh et al. 2020) (Table 2). In other parts of the world, Vitamin E had no effect on milk yield except in one study where cows fed a diet with high plant oil content increased milk production when Vitamin E was added (O'Donnell-Megaro et al. 2012) and in one experiment in Sweden where Vitamin E supplementation reduced milk production in grass-legume based diet (Lindqvist et al. 2011).

Milk lipids are susceptible to oxidation, which leads to undesirable flavour (rancid). Among the oldest studies included here, the effect of Vitamin E on milk lipid oxidative stability or flavour were the focus. More recent studies have also examined the effect of Vitamin E on milk quality, particularly where diets elevated milk fat concentrations of polyunsaturated fatty acids (PUFA). The results are not consistent; some report improved milk quality, i.e. reduced lipid oxidation and off-flavour (Krukovsky and Loosli 1952; De Luca et al. 1957; Dunkley et al. 1967; Stlaurent et al. 1990; Atwal et al. 1991; Nicholson and Stlaurent 1991; Nicholson et al. 1991; Chawla et al. 2003; Al-Mabruk et al. 2004), while many found no effect (Dunkley et al. 1968; Lundin and Palmquist 1983; Charmley et al. 1993; Charmley and Nicholson 1994; Givens et al. 2003; Santos et al. 2019). One study found even more oxidised flavour in milk from Vitamin E supplemented cows (Schingoethe et al. 1979).

In some studies Vitamin E proved to prevent milk fat depression (Charmley and Nicholson 1994; Focant et al. 1998; Kay et al. 2005; Bell et al. 2006; Pottier et al. 2006; Schäfers et al. 2017, 2018

and b), while others found no effect (Lundin and Palmquist 1983; Givens et al. 2003; Weiss and Wyatt 2003; Deaville et al. 2004; O'Donnell-Megaro et al. 2012; Zened et al. 2012; Ramírez-Mella et al. 2013).

### ***State-of-the-art in Vitamin E supplementation and health status in organic dairy cattle production***

Few studies on Vitamin E status of dairy cows directly relate to organic production systems. Blood samples from 5 early lactating dairy cows from each of 14 organic dairy farms, taken during the indoor winter-feeding period, indicated that the Vitamin E status was adequate (Govasmark et al. 2005). The basal diet of the dairy cows in this study was grass clover silage. Blood samples from 10 dairy cows on each of 6 organic dairy farms were analysed in a study conducted in Flanders, Belgium (Beeckman et al. 2010). They concluded that peripartum cows, -50 and 30 days relative to calving, were at risk of suffering from Vitamin E shortage during the indoor feeding period on conserved forages. In a field experiment with dairy cows over 2 complete lactations in Sweden, it was found that high producing dairy cows ( $>9800$  kg energy corrected milk (ECM) cow $^{-1}$  year $^{-1}$ ) received their recommended Vitamin E supply from the diet without any Vitamin E supplementation, except during the peripartum (the period 3 wk before and 3 wk after calving) (Johansson et al. 2014). In another Swedish study, organically managed dairy cows received no additional Vitamin E or were supplemented daily with 2400 IU RRR- $\alpha$ -tocopheryl acetate during the peripartum period (Lindqvist et al. 2011). Supplementation increased the plasma and milk concentration of  $\alpha$ -tocopherol during the transition period, but no difference was found later in lactation. Plasma and milk  $\alpha$ -tocopherol concentrations were higher in mid lactating organically managed cows supplemented with 1900 IU Vitamin than in cows without Vitamin E supplementation in an experiment in Norway (Kidane et al. 2015). However, the plasma concentration was also high in cows without any Vitamin E supplementation ( $>9$   $\mu\text{g ml}^{-1}$ ). Vitamin E supplementation had no effect on antibody response to immunisation, an indicator of adaptive immune status, except for the cows supplemented with RRR- $\alpha$ -tocopheryl acetate that tended to have a lower active immune defence as indicated by the antibody titre values (Kidane et al. 2015). In the other studies on organic management referred to above, no significant differences in animal health or animal health-related parameters were reported.

However, in a long-term study by Johansson et al. (2014), non-supplemented cows tended to have higher milk somatic cell count (SCC) and more cases of mastitis in the second year of the study. This raises the question of whether udder health could be a weak point in organic dairy systems with low supplementation of Vitamin E. Studies comparing udder health in organic and conventional dairy production show contradictory results. Subclinical mastitis was reported to be a greater problem in organic than conventional dairy production in a Swiss study (Roesch et al. 2007), probably due to more restrictive use of antibiotic dry cow therapy in organic than in conventional production. Other studies have found lower incident rates of clinical mastitis on organic than on conventional dairy farms (Hardeng and Edge 2001; Hamilton et al. 2006; Levison et al. 2016). The mechanism for lower mastitis incidence rates in organic than in conventional systems is equivocal. The authors pointed to factors like better animal husbandry skills (Ivemeyer et al. 2009), a broader scope of breeding values, rather than a narrow focus on milk yields (Bludau et al. 2014; Bieber et al. 2019), and a lower plane of nutrition (higher dietary forage:concentrate ratio; European Parliament and the Council of the European Union 2018). Moreover, the organic standards also require that dairy cows have access to pasture for the entire grazing season. Fresh grass has a high content of  $\alpha$ -tocopherol (see next section) and leads to increased Vitamin E concentrations in the milk (Leiber et al. 2005), which might be a reason for the fact that grazing was shown to reduce the risk of mastitis (Firth et al. 2019). Thus, mastitis does not appear to be at a higher incidence

in organic systems, which would increase requirements for Vitamin E during lactation. However, perception and detection of disease may be influenced by the management system, which make comparison between systems difficult (Ruegg 2009).

## **Development of a proposition for organic-specific Vitamin E supplementation**

Based on the findings from the systematic review outlined above, a proposal for Vitamin E dosages for dairy cows in organic production systems was developed. This considers the essentiality of Vitamin E as discussed above, but also the specific nutritional basis for organic dairy production in the European context.

### ***Diets of dairy cows in organic production in Europe***

The results summarised in [Table 3](#) show that fresh grass provides by far the highest concentrations of  $\alpha$ -tocopherol among organic feedstuffs for cattle. Of the conserved feeds, grass clover silage generally contained more  $\alpha$ -tocopherol than hay, whole crop silage, maize silage and cereals used in concentrate mixtures ([Table 3](#)). Losses of  $\alpha$ -tocopherol during storage were minor in grass-legume silages but were about 50% in cereal whole-crop silages (Mogensen et al. 2012).

Diets of dairy cows in organic production in Europe vary due to climate, topography, farm size, economic frame, and regulations. Characterisations of major dairy farm types in some European countries showed that there is a wide range of feeding practices (Wallenbeck et al. 2019). Generally, low concentrate use is aspired to in organic ruminant feeding (European Parliament and the Council of the European Union 2018; Leiber 2022); this is why forage proportions in the diet ranged from 61% to 92%, with farms in Northern Europe having the highest and farms in the alpine region the lowest level of concentrate ([Table 4](#)). Most farms feed grass clover silage and hay during the indoor period ([Table 4](#)), and about 20–30 % of the farms in the Alpine region (Austria (AT), Switzerland (CH) and Germany (DE)) had hay as their main forage (figures not shown). In Northern Europe, grass clover silage was the most important forage source, while haylage is common in the Eastern European countries. Based on these characteristics it is reasonable to assume that the basic provision with Vitamin E from feedstuffs is better in organic rather than conventional dairy systems, which is in line with good tocopherol status in studies on high producing organic dairy cows (Lindqvist et al. 2011; Johansson et al. 2014).

### ***Recommendations for dairy cows in organic production***

National Academies of Sciences Engineering and Medicine (2021) state: ‘Inadequate data are available to determine a requirement for Vitamin E, but based mainly on cow health, an adequate intake for Vitamin E can be established’. In addition, due to the variability of concentration of  $\alpha$ -tocopherol in the basal feed used, the adequate intake is given as supplemental Vitamin E and not total dietary intake. The concentrations of  $\alpha$ -tocopherol in the basal feed used in organic dairy production in Europe also vary considerably ([Table 3](#)), and a recommendation for organic dairy cows must also refer to supplemental Vitamin E. Based on the literature review, the surveys of Vitamin E status on organic dairy farms (Govasmark et al. 2005; Beeckman et al. 2010; Mogensen et al. 2012), the experiments with Vitamin E supplementation conducted on organic dairy farming systems (Lindqvist et al. 2011; Johansson et al. 2014; Kidane et al. 2015), studies on udder health in organic dairy production (Hardeng and Edge 2001; Hamilton et al. 2006; Roesch et al. 2007; Ivemeyer et al. 2012; Levison et al. 2016; Krieger et al. 2017), the diet of major organic farming types following the standards set by the European regulations (European Parliament and the Council of the European Union 2018) and on the expected Vitamin E levels in forages, a proposal for adequate intake for dairy cows was proposed ([Table 5](#)). It was assumed that the selenium supply was adequate, and it was assumed that concentrate proportion in the diet was, according to the

**Table 3.**  $\alpha$ -tocopherol<sup>a</sup>, mg kg<sup>-1</sup> DM, concentration in different conserved or fresh forages, pasture and cereals in organic managed livestock systems. Average values and standard deviations (where applicable) in brackets.

Source	Country/type of study	Grass clover silage	Maize silage	Hay	Haylage	Pasture/fresh grass
Mogensen et al. (2012)	DK/Survey	30 (11.6)	13 (7.7)			
Beeckman et al. (2010)	BE/Survey	52 (35)	4.5 (1.7)	4		
Höjer et al. (2012)	SE&NO/Exp.	27 (7.4)				
Kidane et al. (2015)	NO/Exp.	39 (2.4)				
Adler et al. (2013)	NO/Exp.					79 (7.6)
Lindqvist et al. (2011)	SE/Exp.	45 (26)				30 (10.3)
Johansson et al. (2014)	SE/Exp	58 (17)				36 (9.3)
Jensen (2003)	DK/Review	10–150		5–55	15–65	100–200
Müller et al. (2007)	SE&DK/Exp	37				28
Tian et al. (2020)	CN/Exp	36–39				
Elgersma et al. (2015)	DK/Exp					25–60
Graulet et al. (2012)	FR/Exp					96–123
Maxin et al. (2020)	FR/Exp					34–163

<sup>a</sup>In forages,  $\alpha$ -tocopherol is in the form of the *RRR*-isomer, and Vitamin E in IU can be calculated by multiplying the concentration of  $\alpha$ -tocopherol with a factor of 1.49 or 2.22. The first (1.49) is the conversion factor recommended by the US Food and Drug Administration (FDA 2019), while the second (2.22) is used by the National Academies of Sciences Engineering and Medicine (2021), as data from experiments with dairy cows indicate this.

**Table 4.** Farm feeding and production level for major organic dairy farm types in Europe (adapted from Wallenbeck et al. 2019).

	AT-L	AT-T	CH	DE-L	DE-M	DE-S	DK	LT	PL	SE
Diet										
Concentrate, %	22	14	08	30	17	14	34	21	25	39
Forage, %	78	86	92	70	83	86	66	79	75	61
Indoor season										
Hay, % of farms	69	100	100	36	85	100	90	41	100	16
Haylage, % of farms	0	0	0	0	0	0	20	68	61	3
Silage, % of farms	81	88	60	91	75	70	50	36	0	86
Summer season										
Hay, % of farms	73	94	45	30	40	60	50	5	75	22
Haylage, % of farms	0	0	0	0	0	0	0	10	22	4
Pasture, % of farms	12	18	70	80	85	80	90	95	100	78
Silage, % of farms	45	41	15	60	40	40	10	0	5	44
Milk production										
kg ECM cow <sup>-1</sup> year <sup>-1</sup>	7055	6311	6102	7732	6180	5370	9505	6114	3051	9032
SCC $\times 10^3$	178	136	181	249	227	230	213	239	840	197

AT\_L = Austria loose housing barns.

AT-T = Austria tie stall.

CH = Switzerland.

DE-L = Germany large-scale herds.

DE-M = Germany medium-scale herds.

DE-S = Germany small-scale herds.

DK = Denmark.

LT = Lithuania.

PL = Poland.

SE = Sweden.

SCC = somatic cell count in milk.

standards for organic production, less than 40% of the total dry matter intake on average across the lactation. However, as maize, and whole crop silages are regarded as forage and not concentrate, it was necessary to take their general low concentrations of  $\alpha$ -tocopherol into account. The same was applied to diets where hay or haylage are the main forage sources. The levels proposed with the current approach are similar to the INRA recommendations (INRA 2018), except that it is here suggested to reduce the supplementation to cows on pasture and to differentiate between forage types. The required amounts of Vitamin E during lactation are most likely achieved without supplementation for grazing dairy cows and for cows that are fed grass-clover silage as their

**Table 5.** Proposed dietary recommendation of daily Vitamin E supplementation as IU kg<sup>-1</sup> total dry matter intake (DMI) and as IU kg<sup>-1</sup> body weight (BW), assuming 600 kg BW, for organic dairy cows adjusted for the main forage type in the diet.

DMI kg d <sup>-1</sup>	IU kg <sup>-1</sup> DMI			IU kg <sup>-1</sup> BW		
	Pasture	Grass clover silage	Other conserved forages	Pasture	Grass clover silage	Other conserved forages
Gestating	10	15	25	25	0.3	0.4
Lactating<30 days in milk	15	15	15	25	0.4	0.4
Lactating>30 days in milk	20	0	0	15	0	0.5

main forage source. When needed, Vitamin E supplementation should preferably be administrated as RRR- $\alpha$ -tocopherol acetate, based on extract from vegetable sources, instead of the synthetic all-rac- $\alpha$  tocopherol acetate as it has higher biological availability and closer compliance with the organic standards.

## Conclusions

In order to reach closer to the organic standard claim of not using synthetic molecules in livestock production, it is necessary to revise the Vitamin E supplementation recommendations for dairy cows in organic production systems. This systematic review of the experimental state-of-the-art regarding Vitamin E requirements of dairy cows fed diets with high proportions of forage, including the review of Vitamin E concentrations in various types of fresh and conserved forages, gave a sound background to propose supplementation levels for organic dairy cows under European conditions. Based on the outcomes of the systematic review and in the context of the systematic situation in European organic dairy production, it was proposed that mid and late lactating cows being fed on fresh forage or clover- or grass-silages (respecting the EU allowances for concentrates in organic ruminant diets) do not need to be supplemented with Vitamin E. For peripartum cows and for feeding regimes including maize silage, hay or haylage, a supplementation was, however, strongly recommended. This proposal implies a lower overall Vitamin E supplementation to organic dairy cows if compared with conventional practise.

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