Contrasted effects of dietary extruded linseed supplementation on carotenoid and liposoluble vitamin status in lactating Holstein or Montbéliarde cows fed hay or corn silage

Benoît Graulet,^{1*} Carole Cirié,² and Bruno Martin¹

¹Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, F-63122 Saint-Genès-Champanelle, France

²UE 1414 Herbipôle, INRA, F-63122 Saint-Genès-Champanelle, France

ABSTRACT

Cow milk and dairy products have a good nutritional value that could be improved by increasing the concentrations of several compounds such as carotenoids and liposoluble vitamins A and E. Their concentrations in milk are dependent on their respective dietary intakes, but the transfer from feeds to milk seems to be limited by dietary, digestive, or metabolic factors linked to lipids that could differ between dairy breeds. The effect of dietary fat supplement (provided as extruded linseed) on carotenoid, vitamin E, and vitamin A status as well as their transfer from diet to milk were explored in mid-lactating dairy cows (Holstein or Montbéliarde breed) receiving either corn silage or hay as the main forage. Carotenoid and tocopherol status were higher in cows fed hay than in those fed corn silage, both at the plasma and milk level. The transfer rate for carotenoids was the same regardless of forage, whereas the transfer rate for tocopherols was greater (1.71 vs. 1.20%, respectively) for cows fed hay compared with corn silage. Cows fed extruded linseed had greater plasma concentrations of tocopherols (+25%) compared with those that did not, regardless of forage, but linseed treatment only changed xanthophyll (+35%) concentrations. This would suggest that the lipid supplement increased the availability of xanthophylls and tocopherols for the cows. However, carotenoid transfer into milk remained low and unaffected by the lipid supplement, whatever the forage nature, suggesting a limiting unknown process. Carotenoid status was marginally different between breeds because plasma concentrations were higher in Montbéliarde cows besides lower intakes. In milk, 13-E- β -carotene concentration was also higher for Montbéliarde cows because of a 2-fold higher transfer rate than for Holstein cows. In contrast, Holstein cows had higher transfer rates of α -tocopherol and vitamin

A activity, linked to higher milk fat yield. For the first time, this study proposed an evaluation of the transfer rate of lipid micronutrients from diet to milk in cows. The study highlighted that these compounds follow distinct patterns of regulation during their transfer. However, in these experimental conditions, it was not possible to show that a dietary fat supplement could increase the concentration of these compounds in milk fat.

Key words: dairy cow, milk, vitamin, carotenoid, linseed

INTRODUCTION

Cow milk possesses a nutritional value dependent on its content in nutrients that are proteins (Kukovics and Németh, 2013; Korhonen and Marnila, 2013; Bernard et al., 2018), lipids (Gordon, 2013), lactose and oligosaccharides (Crisa, 2013), minerals (Gaucheron, 2013), specific fatty acids (butyrate, branched-chain, conjugated; Bernard et al., 2018), and vitamins and carotenoids (Nozière et al., 2006; Graulet et al., 2013; Bernard et al., 2018). Among them, the content and composition in lipid compounds (lipid classes, fatty acids, vitamins A and E, carotenoids) depend largely on the nature of the diet ingested by the cows (Bernard et al., 2018). Consequently, the search of improvement of the nutritional quality of products led to the development of programs using feed management of cows to control the composition of the fat-soluble fraction in milk and dairy products. For decades, attempts mainly concerned milk fatty acid composition and studies on carotenoids and vitamins were less common, besides their nutritional and sensorial interests as antioxidants, pigments for vision, and regulators of major functions (reproduction, growth, immunity, and so on; Graulet et al., 2013). Nevertheless, regarding β -carotene, experimental data have indicated a large variability in its cow milk concentration, resulting from intrinsic (breed, lactation stage, parity, health, milk performances, and genetics) and extrinsic factors (mainly diet composi-

Received December 10, 2018.

Accepted March 5, 2019.

*Corresponding author: benoit.graulet@inra.fr

tion; Nozière et al., 2006). Indeed, experiments performed in controlled conditions (Calderon et al., 2007) and observations from commercial farms (Agabriel et al., 2007) demonstrated that milk concentrations of β -carotene and α -tocopherol increased with their respective intakes, in a dose-response manner (Calderon et al., 2007). It explained why the highest concentrations of β -carotene (until 13.9 $\mu\text{g/g}$ of milk fat) are found in milk of cows fed on fresh pastures during spring because they are the highest dietary sources of β -carotene for ruminants (Nozière et al., 2006). By comparison, diets based on hay or corn silage induce the production of milks largely poorer in carotenoids (1.5 to 4.7 $\mu\text{g/g}$ of milk fat; Nozière et al., 2006). These latter rearing conditions could lead to sensorial issues for dairy industry because of the whiteness of milk fat (highlighted in cheeses and butters) that would not meet consumers' expectations, and of the development of off-flavors resulting from lipid oxidation, especially the PUFA, which are highly oxygen sensitive.

Even though the response in term of concentrations in milk is proportional to intakes for these compounds, the transfer from diet to milk does not seem to be very efficient. Data are lacking or sometimes there is a lack of consistency, especially in ruminants, to identify clearly the critical steps in this transfer (review in Nozière et al., 2006). However, carotenoid and tocopherol availability and metabolism are linked to lipids: absorption processes require lipids in the diet, blood transport is performed through lipoproteins, tissue storage is linked to lipid compartments such as fat droplets for β -carotene or lipid biomembranes for tocopherols, and excretion into milk is associated with the fat globule (Brown et al., 2004; Reboul, 2017; Bernard et al., 2018). Thus, the level of dietary fat could be a limiting factor for the bioavailability of these compounds, especially in ruminants for which diet is usually rather poor in lipids. Indeed, in humans, Brown et al. (2004) observed a positive relationship between the level of dietary fat and the plasma carotenoid concentration. Moreover, Puppel et al. (2016) observed recently that dietary n-3-rich fat supplements increase the β -carotene (2.45-fold), vitamin A (+17%), and vitamin E (+43%) concentrations in the milk of cows in mid-lactation fed a TMR based on a mixture of corn and alfalfa silages. It was also observed that rumen-protected CLA distribution in the diet around calving could specifically stimulate the secretion of retinol (+34%) and tocopherols (+44% for α and +21% for γ) into milk of cows during the first 14 wk of lactation (Gessner et al., 2015).

In the present work, the aim was to increase carotenoid, tocopherol, and vitamin A transfer from diet to milk in dairy cows through a dietary fat supplement, given as extruded linseed (**EL**). This effect was com-

pared in 2 distinct dietary conditions (a forage based on maize silage plus some hay vs. a forage made of hay alone) to see if the nature of the diet and the type of rumen fermentations could influence the process. Moreover, because the lipid metabolism and the richness of milk in these vitamins can vary between breeds, the comparison was also made between Holstein (**HO**) and Montbéliarde (**MO**) cows. Through this comparison, we aimed to compare the efficiency of carotenoid and vitamin transfer to milk and to evaluate if a dietary lipid supplementation had the same effects on carotenoid and vitamin status between the 2 bovine species.

MATERIALS AND METHODS

Animals and Diets

Twenty-four lactating cows of the experimental herd of Marcenat (INRA, Herbipole, France) were used for the experiment. This herd was half HO and half MO breeds. Among each breed, 8 cows were multiparous and 4 were primiparous. During a 3-wk pre-experimental period, each cow received daily a diet based on hay (4.7 kg of DM/d) and maize silage ad libitum (9.4 kg of DM/d as a mean) plus an individual concentrated mixture made of cereals (70% barley and 30% maize; maximum 4.6 kg) and soybean meal (around 2.7 kg/d), according to individual milk production. Then, cows were assigned to 1 of 4 groups of 6 cows balanced for breed, parity, lactation number (2.6 ± 1.6), DIM (63.0 ± 0.6 d), milk yield (31.3 ± 5.7 kg/d), and BW (653 ± 58 kg; mean $\pm$ SD) at the end of the pre-experimental period.

After a 3-d adaptation period, cows received 1 of 4 experimental diets for a 5-wk experimental period (Table 1). Two diets were based either on corn silage offered ad libitum (13.8 kg of DM/d as a mean) plus 1.7 kg of DM/d of natural grassland hay (**CS** diet) or natural grassland hay dried in barn as the only forage (13.5 kg of DM/d as a mean; **H** diet). Cows ingested a ration corresponding to their needs so that micro-nutrient intakes, plasma levels, milk secretion, and transfer rates were evaluated as reference physiological values that should be used accordingly. The CS and H cows received an average amount of 2.2 kg of DM/d of ground barley and corn (70%/30%) and 2.6 kg of DM/d of solvent-extracted soybean meal, which were adjusted weekly to maintain a 70:30 (DM basis) forage to concentrate ratio during the experimental period. The 2 other groups received the H or CS diets in which an extruded blend of 70:30 wt/wt linseeds and wheat (Neovia, Argentan, France) at a 2.5% of additional oil in DMI was given in substitution to the concentrate mixture (**CSEL** and **HEL** diets, respectively). Energy

Table 1. Composition of experimental diets¹

Diet composition, g/kg of DM	CS	CSEL	H	HEL
Corn silage	642.6	646.0	0.0	0.0
Natural grassland hay	80.3	79.9	0.0	0.0
Natural grassland hay dried in barn	0.0	0.0	564.0	602.0
Barley and corn mix	140.8	64.0	365.0	271.5
Extruded linseed	0.0	88.2	0.0	79.8
Soybean meal	127.5	113.1	62.5	38.9
Mineral and vitamin complement	8.8	8.7	8.5	7.8

¹CS = diet composed of corn silage as predominant forage and not containing extruded linseed; CSEL = diet composed of corn silage as predominant forage and containing extruded linseed; H = diet composed of hay as predominant forage and not containing extruded linseed; HEL = diet composed of hay as predominant forage and containing extruded linseed.

and protein dietary intakes covered at least 110% of the individual energy and protein requirements (INRA, 2007). All cows received 200 g/d of mineral and vitamin premix containing 5 g/kg of P, 20 g/kg of Ca, 4.5 g/kg of Mg, 2 g/kg of Na, 1 g/kg of S, 600,000 IU/kg of vitamin A, 120,000 IU/kg of vitamin D₃, 1,500 IU/kg of vitamin E, 400 mg/kg of vitamin B₁, 1,400 mg/kg of Cu, 5,000 mg/kg of Zn, 4,000 mg/kg of Mn, 80 mg/kg of I, 32 mg/kg of Co, and 22 mg/kg of Se.

Cows were housed in a tiestall barn and were milked at 0600 and 1600 h in a milking parlor. Diet refusals were collected and weighed daily 4 d per week to calculate the real intake of each cow during the experiment. Cow BW and BCS were recorded at the end of the pre-experimental and experimental periods. The protocol was approved by the Institut National de la Recherche Agronomique Animal Care and Use Committee. All procedures were conducted in accordance with French Guidelines for the use of experimental animals compliant with animal welfare and good practice (Veissier, 1999) at the time of the experiment. The Herbipole facilities had agreement for animal experimentation C6334517.

Samplings, Measurements, and Analyses

Feedstuffs. Samples of corn silage, concentrates, hays, and feed refusals were collected once during the last week of the pre-experimental period, and once per week during the last 3 wk of the experimental period. The 3 samples obtained during experimental period were pooled to provide one sample per period and stored at -20°C until analysis. Feed DM concentration was determined after drying at 104°C for 24 h. Samples of corn silage, hay, and concentrates were lyophilized, sieved through a 1-mm screen, and analyzed for CP, crude fiber, NDF, ADF, starch, ether extract, and OM using standard procedures (AOAC International, 1997). The net energy value calculated using equations from INRA (2007) and OM digestibility was 6.11 (MJ/kg of

DM). Carotenoid and tocopherol analysis in feedstuffs were performed as described by Chauveau-Duriot et al. (2010). Briefly, the method was based on organic solvent extraction, saponification to eliminate chlorophyll esters, then final purification before chromatographic analysis with a Acquity UPLC system (Waters, Saint-Quentin-en-Yvelines, France) using a 150×2.1 mm, HSS T3, $1.8\text{-}\mu\text{m}$ column. The method originally developed for forages (mainly grass-based forages) was also used successfully for the other feedstuffs of the diets, increasing the sample size to 300 mg of DM for concentrates (soybean meal, cereal mixture, and EL) and 200 mg of DM for corn silage. The characteristics of feedstuffs including their contents in carotenoids and tocopherols are given in Table 2.

Blood. Blood samples were collected from the jugular vein in the 4th day of the last week of the pre-experimental or experimental periods, from each cow, before the morning feeding, in tubes containing EDTA (2.1 mg/mL). Plasma was separated by centrifugation immediately after sampling at $1,500 \times g$ for 15 min at room temperature. Plasma samples were frozen at dark at -20°C until analyzed. Carotenoid, retinol, and tocopherol analysis in plasma were performed with a Acquity UPLC system (Waters) using a 150×2.1 mm, HSS T3, $1.8\text{-}\mu\text{m}$ column as described by Chauveau-Duriot et al. (2010).

Milk. Daily milk yield was recorded individually. Milk fat, protein, and lactose percentages and SCC were determined for individual cows from 4 consecutive milkings each week. For this, one sub-sample (50 mL) was preserved in tubes with bronopol-B₂ (Trillaud, Surgères, France) and stored at 4°C for analysis. Milk fat and protein percentages were assayed (Galilait, Theix, France) by standard procedures (NIRS, Milkoscan 4000, Foss Electric, Hillerød, Denmark; AOAC International, 1997). Other milk samples representative of individual daily production were obtained by mixing milks of each cow and each sampling period from morning and evening milkings in 60/40 proportions. Samples

Table 2. Characteristics of the experimental feedstuffs

Item	Corn silage	Natural grassland hay	Natural grassland hay dried in barn	Barley and corn mix	Extruded linseed	Soybean meal
DM, ¹ g/kg	320	850	850	865	900	878
OM, ² g/kg of DM	955	925	927	981	971	933
CP, ³ g/kg of DM	91	97	106	97	209	522
Crude fiber, ⁴ g/kg of DM	186	327	280	47	88	66
Fat, ² g/kg of DM	53	26	13	44	267	18
NDF, ² %	46.4	65.2	58.2	187.2	20.1	15.5
ADF, ² %	24.2	2.6	31.3	53.1	8.9	7.4
PDIN, ^{5,6} g/kg of DM	47	72	66	75	166	377
PDIE, ^{5,7} g/kg of DM	67	75	88	98	144	261
UFL, UF/kg of DM	0.86	0.62	0.83	1.18	1.60	1.21
Carotenoid content, ⁸ µg/g of DM						
Violaxanthin	0.0	10.0	9.5	0.0	0.0	0.0
Neoxanthin	0.0	12.0	12.5	0.0	0.0	0.0
Antheraxanthin	0.0	0.0	2.6	0.0	0.0	0.0
Lutein epoxide	0.0	0.0	0.9	0.0	0.0	0.0
Lutein	23.5	66.5	83.5	8.5	0.0	5.0
Zeaxanthin	8.2	9.6	17.9	5.0	0.0	0.0
β-Cryptoxanthin	1.8	1.2	0.0	0.0	0.0	0.0
13-Z-β-carotene	0.7	0.0	2.7	0.0	0.0	0.0
9-Z-β-carotene	1.6	2.5	3.7	0.0	0.0	0.0
All-E-β-carotene	12.2	19.1	19.5	3.8	0.0	2.6
Sum of xanthophylls	33.5	99.3	126.9	13.5	0.0	5.0
Sum of carotenes	14.5	21.6	25.9	3.8	0.0	2.6
Sum of carotenoids	49.0	120.9	152.8	17.3	0.0	7.6
Tocopherol content, ⁸ µg/g of DM						
γ-Tocopherol	38.1	40.6	21.5	39.5	92.1	31.1
α-Tocopherol	53.9	66.5	39.1	38.7	46.7	35.9
Sum of tocopherols	92.0	107.0	60.6	78.1	138.7	67.0

¹DM was determined by drying at 104°C for 24 h.

²OM, fat, NDF, and ADF were determined by near-infrared spectroscopy (Galilait, Clermont-Ferrand, France).

³CP was determined by the Kjeldahl method (AOAC International, 1997).

⁴Crude fiber was determined by the Wende method (AOAC International, 1997).

⁵Net energy and nitrogen values were calculated according to INRA (2007) guidelines.

⁶PDIN = proteins digestible in the intestine, when degraded N is a limiting factor.

⁷PDIE = proteins digestible in the intestine, when energy is a limiting factor.

⁸Carotenoids and tocopherols were determined according to Chauveau-Duriot et al. (2010).

were stored at −20°C at dark until analysis. Carotenoid, retinol, and tocopherol analyses were performed with a Acquity UPLC system (Waters) using a 150 × 2.1 mm, HSS T3, 1.8-µm column as described by Chauveau-Duriot et al. (2010).

Calculations and Statistical Analyses

The intake and the chemical composition (including carotenoids and tocopherols) of each feedstuff were used to calculate the total intakes. Moreover, concentrations in carotenoids, retinol, and tocopherols in individual milks as well as daily milk yields were used to calculate the total amount secreted per cow per day for each carotenoid, retinol, and tocopherol. Finally, a transfer rate from diet to milk for each cow was determined by reporting the amount secreted per day to the amount ingested per day, for each compound.

Data were analyzed by ANOVA using a statistical model that included the nature of the forage in the

diet (**F**), the supplementation or not with EL (**L**), the breed (**B**), as well as their 2-way and 3-way interactions as fixed effects with Minitab 18 (Minitab Inc., Paris, France). For each parameter, a covariate term was obtained from the deviation between the individual value and the mean for the corresponding breed at the end of the pre-experimental period.

RESULTS

Dietary Intake and Milk Performances

The mean values of the intakes of DM and its components, as well as energy and protein balances, milk production, and composition are given in Table 3.

The DM, OM, fiber, and net energy for milk production (**UFL**) intakes were higher in H compared with CS diets, whereas CP, fat, and proteins digestible in the intestine when degraded N is a limiting factor (**PDIN**) were lower. For both forages, DM, OM, CP, crude fiber,

Table 3. Performances of Holstein (HO) or Montbéliarde (MO) cows fed diets based on hay or corn silage with or without extruded linseed supplement for 5 wk

Item	Corn silage				Hay				ANOVA <i>P</i> -value ¹			
	EL+		EL−		EL+		EL−		SEM	F	L	B
	HO	MO	HO	MO	HO	MO	HO	MO				
BW, kg	659.6	656.6	660.0	661.2	656.2	657.8	657.6	645.7	9.5	0.216	0.716	0.443
BCS	1.5	2.0	1.5	2.0	1.4	1.9	1.5	2.1	0.2	0.719	0.545	<0.001
DML, kg of DM/d	24.0 ^{ab}	18.9 ^c	23.5 ^{ab}	18.8 ^c	26.3 ^a	21.9 ^{bc}	22.4 ^b	22.0 ^b	1.1	0.001	0.028	<0.001
OM, kg of DM/d	22.8 ^{ab}	17.8 ^c	22.3 ^{ab}	17.7 ^c	24.7 ^a	20.6 ^{bc}	21.1 ^b	20.7 ^{bc}	1.0	0.002	0.030	<0.001
CP, kg of DM/d	3.67 ^a	2.82 ^b	3.42 ^a	2.79 ^b	3.32 ^a	2.78 ^b	2.86 ^b	2.81 ^b	0.15	0.001	0.009	<0.001
Crude fiber, kg of DM/d	3.76 ^{bc}	3.24 ^c	3.61 ^{bc}	3.20 ^c	5.00 ^a	4.19 ^{ab}	4.04 ^{bc}	3.95 ^{bc}	0.30	<0.001	0.013	0.002
NDF, kg of DM/d	8.85 ^b	7.48 ^b	9.03 ^{ab}	7.80 ^b	10.77 ^a	8.95 ^b	9.17 ^{ab}	8.96 ^b	0.61	<0.001	0.297	<0.001
ADF, kg of DM/d	4.53 ^{ab}	3.93 ^b	4.55 ^{ab}	4.05 ^b	5.43 ^a	4.53 ^{ab}	4.54 ^{ab}	4.43 ^b	0.34	0.005	0.151	0.002
Fat, kg of DM/d	1.54 ^a	1.24 ^b	1.05 ^{bc}	0.84 ^d	1.07 ^{bc}	0.95 ^{cd}	0.55 ^e	0.54 ^e	0.07	<0.001	<0.001	<0.001
UFL, kg/d	23.3 ^{ab}	17.6 ^c	21.9 ^b	16.8 ^c	26.2 ^a	21.8 ^b	21.8 ^b	21.4 ^b	1.0	<0.001	0.001	<0.001
PDIN, g/d	2,550 ^a	1,932 ^b	2,343 ^a	1,891 ^b	2,299 ^a	1,925 ^b	1,968 ^b	1,940 ^b	99	0.003	0.003	<0.001
PDIE, g/d	2,624 ^a	2,032 ^{cd}	2,477 ^{abc}	1,973 ^d	2,568 ^{ab}	2,114 ^{bcd}	2,185 ^{abcd}	2,144 ^{abcd}	175	0.746	0.072	<0.001
Balance ^a												
UFL, UFL/d	0.71	0.99	1.11	0.14	2.71	2.25	1.11	2.82	1.06	0.004	0.416	0.745
PDIN, g/d	274	253	321	247	−32	−116	−84	54	104	<0.001	0.365	0.811
PDIE, g/d	338 ^{ab}	306 ^{ab}	457 ^a	310 ^{ab}	230 ^{ab}	102 ^b	147 ^b	293 ^{ab}	106	0.003	0.202	0.372
Milk yield, kg/d	36.8 ^{ab}	25.5 ^{de}	30.4 ^{cd}	23.5 ^e	39.4 ^a	30.2 ^{cd}	32.1 ^{bc}	27.6 ^{cde}	1.9	0.001	<0.001	<0.001
Fat content, g/kg	38.5 ^{ab}	35.5 ^{ab}	44.1 ^a	41.7 ^{ab}	35.3 ^{ab}	34.9 ^b	37.4 ^{ab}	35.1 ^{ab}	3.2	0.005	0.017	0.144
Protein content, g/kg	32.4	31.9	33.8	34.2	31.7	34.1	33.1	33.5	1.0	0.999	0.017	0.132
Lactose content, g/kg	51.6	51.8	50.9	50.6	53.7	55.1	52.4	52.9	1.1	0.002	0.017	0.394
Fat yield, g/d	1,428 ^a	896 ^d	1,333 ^{ab}	961 ^{cd}	1,347 ^{ab}	1,063 ^{cd}	1,175 ^{bc}	970 ^{cd}	88	0.663	0.058	<0.001
Protein yield, g/d	1,185 ^{ab}	808 ^d	1,022 ^{bc}	796 ^d	1,226 ^a	1,036 ^{bc}	1,050 ^{bc}	929 ^{cd}	59	0.001	<0.001	<0.001

^{a-c}Means (*n* = 3) within a row not sharing a common superscript differ (*P* < 0.05) due to interactions between forage nature, lipid supplement, or cow breed, or all 3.

¹Probability of significant effects due to the nature of the forage (F), the presence (EL+) or not (EL−) of extruded linseed in the diet (L), the breed of the cows (B), and their interactions (F × L, F × B, L × B, and F × L × B).

²Balances were calculated as the difference between intakes and production requirements (INRA, 2007); UFL = net energy for milk production; PDIN = protein digestible in the intestine when degraded N is a limiting factor; PDIE = protein digestible in the intestine when energy is a limiting factor.

fat, UFL, and proteins digestible in the intestine (**PDI**) intakes were slightly higher when EL was added to the diets. The DM, OM, CP, crude fiber, fat, UFL, and PDI intakes were also higher in HO than MO breeds, except in the hay diet without EL where MO and HO intakes were similar, which resulted in significant $F \times B$ and $L \times B$ interactions.

The UFL and PDI balances differed according to the nature of the forage but not according to breed or EL supplementation. Cows fed hay had a higher positive UFL balance than cow fed corn silage, whereas the opposite was observed for proteins digestible in the intestine when energy is a limiting factor, and PDIN. The PDIN balance was slightly negative for hay fed cows (-45 g/d), whereas it was positive for cows fed corn silage ($+274$ g/d).

Milk yield was lower in cows fed corn silage than in cows fed hay as the sole forage (-10% , $P = 0.001$). Moreover, in average, HO cows produced 30% more milk than MO cows, especially when HO cows received EL in their diet (38.1 kg/d) whereas the distribution of EL had no effect in MO cows (P -value of the $L \times B = 0.011$). The milk gross composition was different according to forage nature, lipid supplementation but not to cow breed. Indeed, the fat content was higher in cows fed corn silage ($+12\%$, $P = 0.005$), and lower in cows fed EL (-2.7% , $P = 0.017$), especially when forage is corn silage (P -value of the $F \times L = 0.098$). Lipid distribution slightly decreased protein content (-3.5% , $P = 0.017$). The lactose content was higher when cows were fed hay ($+4.5\%$, $P = 0.002$) or received EL ($+2.7\%$, $P = 0.017$). Moreover, HO cows had higher fat yield than MO cows, especially when fed corn silage ($+7.8\%$, P -value of the $F \times B = 0.013$). Finally, the protein yield was higher in cows fed hay ($+11\%$, $P = 0.001$), in cows receiving EL, especially of the HO breed ($+18\%$, P -value of the $L \times B = 0.040$) and was notably lower in MO cows fed corn silage (-17.5% , P -value of the $F \times B = 0.013$). The BW (Table 3) and the SCC (data not shown) were not different between groups. The BCS (Table 3) was higher for MO than HO cows ($+35\%$, $P < 0.001$).

Micronutrient Content in Feedstuffs and Intakes

Hays were the richest in carotenoids (between 120 and 160 $\mu\text{g/g}$ of DM), especially as the result of their relative richness in xanthophylls (Table 2). Indeed, the comparison between feedstuffs showed that some xanthophylls (violaxanthin, neoxanthin, antheraxanthin, and lutein epoxide) were only observed in hay and not in other feedstuffs. Moreover, lutein, which was quantitatively the major carotenoid in feedstuffs, was more concentrated in hays. Extruded linseeds did

not contain carotenoids, whereas cereals and soybean meal contained less than 20 $\mu\text{g/g}$ of DM. In contrast, all feedstuffs contained both α - and γ -tocopherols. Their relative proportions were close to one-third of γ -tocopherol and two-thirds of α -tocopherol whatever the forage. In contrast, in the cereal mix and in soybean meal, the proportions were equivalent between the 2 tocopherols. Finally, the γ -tocopherol was dominant (66%) in EL that were also the richest feedstuff.

The average daily intake of each carotenoid and tocopherol, as well as retinol equivalent, per cow, determined experimentally, are given in Table 4. Dietary carotenoid, tocopherol, or retinol equivalent intakes were significantly different ($P < 0.001$) for all these compounds according to forage nature. The sum of carotenoid intake was more than 2-fold higher in hay-fed than corn silage-fed cows, resulting from higher values for all carotenoids except β -cryptoxanthin that was almost lacking in the diet of hay-fed cows. The effect of EL addition in the diet on carotenoid intake was limited to a moderate increase in cows fed hay as sole forage and not when fed corn silage (P -value of the $F \times L = 0.026$ for zeaxanthin to 0.083 neoxanthin), except for β -cryptoxanthin. Consequently, retinol equivalent intake was marginally higher ($+0.2\%$) when cows were fed hay because of the higher intake of β -carotenes, the main precursors of vitamin A. As DMI was significantly lower in MO than in HO cows (Table 3), carotenoid, retinol equivalent and tocopherol intakes were consequently lower for the MO cows: -11% for the carotenoid sum ($P = 0.015$) and -13% for the tocopherol sum ($P < 0.001$). Additionally, tocopherol intake was higher in corn silage-fed than hay-fed cows ($+14$ to $+19\%$, $P < 0.001$), especially in HO cows (P -value of $F \times B < 0.005$). As expected, tocopherol intake was significantly higher when cows received EL resulting both from γ -tocopherol ($+18\%$, $P < 0.001$) and α -tocopherol ($+5\%$, $P = 0.021$) intakes. However, the difference was more marked in HO than in MO cows (P -value of $L \times B < 0.10$).

Plasma Micronutrient Concentrations

Retinol concentration (mean value at 0.83 mg/L) and total vitamin A activity (mean value at 3.52 IU/L) in plasma did not differ according to the factors tested (Table 5). However, the forage composition in the diet had a marked effect on all-E- β -carotene (main carotenoid in plasma and retinol precursor) because cows fed hay had a 2.2-fold higher concentration than cows fed corn silage ($P < 0.001$). The same observation could be made for lutein (4.7-fold, $P < 0.001$), zeaxanthin (3.7-fold, $P = 0.061$), 13-Z- β -carotene ($+50\%$, $P = 0.012$), and xanthophyll, carotene, and carotenoid

Table 4. Dietary intakes of carotenoids, vitamin A, and vitamin E in Holstein (HO) or Montbéliarde (MO) cows fed diets based on hay or corn silage with or without extruded linseed supplement for 5 wk

Item	Corn silage				Hay				ANOVA <i>P</i> -value ¹							F × L × B	
	EL+		EL−		EL+		EL−		SEM	F	L	B	F × L	F × B	L × B		
	HO	MO	HO	MO	HO	MO	HO	MO									
Carotenoid, mg/cow per d																	
Neoxanthin	21 ^b	21 ^b	21 ^b	21 ^b	195 ^a	164 ^a	159 ^a	153 ^a	16	<0.001	0.083	0.175	0.083	0.175	0.364	0.364	0.364
Violaxanthin	17 ^b	16 ^b	17 ^b	17 ^b	149 ^a	125 ^a	121 ^a	117 ^a	12	<0.001	0.084	0.164	0.072	0.168	0.292	0.292	0.328
Lutein	490 ^c	425 ^c	499 ^c	443 ^c	1,383 ^a	1,156 ^{ab}	1,142 ^{ab}	1,110 ^b	89	<0.001	0.094	0.019	0.047	0.356	0.179	0.221	0.221
Zeaxanthin	147 ^c	122 ^c	153 ^c	128 ^c	322 ^a	267 ^b	270 ^b	263 ^b	17	<0.001	0.147	0.001	0.026	0.674	0.112	0.112	0.113
β-Cryptoxanthin	28.6 ^a	25.6 ^a	28.1 ^a	25.7 ^a	0.2 ^b	0.0 ^b	0.0 ^b	0.3 ^b	2	<0.001	0.942	0.140	0.916	0.136	0.788	0.954	0.954
9-Z-β-carotene	27 ^c	24 ^c	26 ^c	25 ^c	59 ^a	49 ^{ab}	48 ^{ab}	46 ^b	4	<0.001	0.055	0.034	0.051	0.382	0.185	0.282	0.282
13-Z-β-carotene	24 ^c	21 ^c	24 ^c	22 ^c	43 ^a	36 ^{ab}	35 ^{ab}	34 ^b	3	<0.001	0.056	0.019	0.056	0.577	0.176	0.295	0.295
All-E-β-carotene	227 ^{bc}	192 ^c	231 ^{bc}	202 ^c	337 ^a	284 ^{ab}	285 ^{ab}	277 ^{ab}	21	<0.001	0.220	0.003	0.053	0.944	0.165	0.256	0.256
Xanthophyll sum	702 ^c	607 ^c	717 ^c	635 ^c	2,106 ^a	1,759 ^{ab}	1,738 ^{ab}	1,688 ^b	135	<0.001	0.092	0.021	0.046	0.337	0.184	0.218	0.218
Carotene sum	278 ^{cd}	238 ^d	282 ^{cd}	249 ^d	439 ^a	369 ^{ab}	367 ^{ab}	358 ^{bc}	28	<0.001	0.157	0.005	0.052	0.892	0.166	0.261	0.261
Carotenoid sum	980 ^c	845 ^c	999 ^c	883 ^c	2,545 ^a	2,129 ^{ab}	2,105 ^{ab}	2,046 ^b	162	<0.001	0.100	0.015	0.045	0.412	0.177	0.223	0.223
Vitamin A, μg/cow per d																	
Retinol equivalent ²	3,628 ^{bc}	3,625 ^c	3,628 ^{bc}	3,625 ^c	3,640 ^a	3,634 ^{ab}	3,634 ^{ab}	3,633 ^b	2	<0.001	0.159	0.004	0.054	0.939	0.166	0.265	0.265
Tocopherol, mg/cow per d																	
α-Tocopherol	1,483 ^a	1,255 ^{cd}	1,443 ^{ab}	1,240 ^{cd}	1,312 ^{bc}	1,141 ^d	1,143 ^d	1,128 ^d	56	<0.001	0.021	<0.001	0.186	0.018	0.068	0.174	0.174
γ-Tocopherol	1,009 ^a	794 ^{bcd}	880 ^b	697 ^{de}	842 ^{bc}	715 ^{cde}	638 ^e	628 ^e	44	<0.001	<0.001	<0.001	0.371	0.003	0.057	0.266	0.266
Tocopherol sum	2,492 ^a	2,049 ^{bcd}	2,323 ^{ab}	1,938 ^{cde}	2,154 ^{bc}	1,856 ^{de}	1,781 ^{de}	1,756 ^e	99	<0.001	<0.001	<0.001	0.249	0.007	0.060	0.205	0.205

^{a-c}Means (*n* = 3) within a row not sharing a common superscript differ (*P* < 0.05) due to interactions between forage nature, lipid supplement, or cow breed, or all 3.
¹Probability of significant effects due to the nature of the forage (F), the presence (EL+) or not (EL−) of extruded linseed in the diet (L), the breed of the cows (B), and their interactions (F × L, F × B, L × B, and F × L × B).
²The vitamin A activity includes the respective vitamin A activities from retinol of the mineral and vitamin complement, β-carotenes, and β-cryptoxanthin of other feedstuffs.

sums ($P < 0.001$). Hay feeding also induced higher plasma α -tocopherol concentration (+28%, $P = 0.037$) and tended to induce a higher plasma tocopherol sum (+22%, $P = 0.078$). Dietary EL increased lutein and β -cryptoxanthin plasma concentrations and the sum of xanthophylls (+35%, $P = 0.006$). However, as illustrated by the 3-way interaction, this difference did not apply to MO cows fed corn silage (P -value of $F \times L \times B = 0.038$). Moreover, as their relative contribution to total plasma carotenoids was moderate, it did not affect total carotenoid concentration. Dietary EL also increased plasma γ -tocopherol concentration (+20%, $P = 0.043$) and tocopherol sum (+25%, $P = 0.044$). The plasma γ -tocopherol concentration was also higher in HO than in MO cows (+22%, $P = 0.023$). The same percentage difference was observed between breeds for α -tocopherol (not significant) and the sum of tocopherols ($P = 0.101$). Plasma carotenoid concentrations were higher in MO than in HO cows, especially for 13-Z- β -carotene, 9-Z- β -carotene (+38 and +125%, respectively, $P < 0.05$), and the sums of carotenes, xanthophylls, and carotenoids (+29, +25, and 29%, respectively, $P < 0.10$). It should be noted that violaxanthin, neoxanthin, lutein epoxide, and antheraxanthin were never observed in plasma of the cows, even at the trace level.

Milk Micronutrient Concentrations

The cows fed hay as main forage had 1.9-fold higher lutein ($P = 0.040$) and total xanthophylls ($P = 0.056$), and 1.3-fold higher total carotenoid ($P = 0.024$) concentrations, but tended to have lower retinol (−29%, $P = 0.059$) concentrations in milk (expressed in mg/L) than cows fed corn silage (Supplemental Table S1; <https://doi.org/10.3168/jds.2018-16138>). Extruded linseed addition in the diet induced a reduction in all-E- β -carotene (−24%, $P = 0.043$), sums of β -carotene (−23%, $P = 0.050$), and carotenoid (−20%, $P = 0.077$) concentrations in milk expressed in milligrams per liter (Supplemental Table S1; <https://doi.org/10.3168/jds.2018-16138>). Moreover, dietary EL also led to milks with less α -tocopherol (−26%) when cows were fed hay as the sole forage but not when they were fed corn silage, values being intermediate in these conditions (P -value of the $F \times L = 0.031$). Additionally, α -tocopherol concentration was higher in the milk of HO than MO cows (+21%, $P = 0.004$). However, γ -tocopherol milk concentration was the highest for hay-fed MO cows (0.480 mg/L) and the lowest in hay-fed HO cows and CS-fed MO cows (0.334 and 0.326 mg/L, respectively), values being intermediate for HO cows fed CS (0.410 mg/L; P -value of the $F \times B = 0.030$; Supplemental Table S1; <https://doi.org/10.3168/jds.2018-16138>).

When related to milk fat, retinol concentration and total vitamin A activity in milk were not different according to the factors tested (Table 6). For other parameters, the main differences were observed between the 2 types of forage. Cows fed a diet where hay was the sole forage had higher concentrations of lutein (+102%, $P = 0.026$), all-E- β -carotene (+36%, $P = 0.044$), xanthophylls (+106%, $P = 0.032$), β -carotenes (+34%, $P = 0.048$), carotenoids (+51%, $P = 0.005$), α -tocopherol (+13%, $P = 0.084$), and tocopherol sum (+19%, $P = 0.058$) in milk fat. Linseed supplement had no effect on all these parameters. The carotenoid concentrations were numerically higher in MO cows than in HO cows but the differences only reached significance for β -cryptoxanthin (4.75-fold, $P < 0.001$) and 13-Z- β -carotene (4.0-fold, $P = 0.012$). Conversely, α -tocopherol concentration tended to be higher in the milk of HO than MO cows (+14%, $P = 0.067$). Interactions were generally not significant, but for milk γ -tocopherol concentration, the difference was statistically significant between MO cows fed corn silage or only hay (respectively 13.9 vs. 7.99 mg/g of milk fat), whereas it was not significant for HO cows (P -value of the $F \times B = 0.021$).

Apparent Transfer Rates of Carotenoids, Vitamin A, and Vitamin E from Diet to Milk

The apparent transfer rates are given in Table 7. They were extremely low for xanthophylls (0.12% for zeaxanthin, 0.16% for lutein, and 0.13% for their sum). For lutein, the transfer rate varied from 0.24% in HO cows of the CSEL group and MO cows of the CS group to 0.06% in HO cows of the CS group (P -value of the $F \times L \times B = 0.076$). For zeaxanthin, the apparent transfer rate tended to be higher for cows supplemented with EL when fed hay but it was not observed in cows fed corn silage (P -value of the $F \times L = 0.096$).

For 13-Z- β -carotene, the apparent transfer rate was significantly higher in MO than in HO cows (2.3-fold, $P < 0.05$). However, the average minimum and maximum transfer rates were observed between MO cows of the CSEL group (1.79%) and HO cows of the H (0.32%) or CSEL (0.19%) groups (P -value of the $F \times L \times B = 0.068$). Among carotenoids, the highest transfer rate was observed for all-E- β -carotene but it was not significantly different between groups (mean value 2.31%). Whereas there was no significant difference for all-E- β -carotene and β -carotene sum, the total carotenoid transfer rate was higher when cows were fed corn silage than when fed hay alone (0.65 vs. 0.44%, $P = 0.002$).

For total vitamin A activity, the transfer rate was not different according to forage or linseed supplement

Table 5. Plasma concentrations (mg/L) of carotenoids, vitamin A, and vitamin E of Holstein (HO) or Montbéliarde (MO) cows fed diets based on hay or corn silage with or without extruded linseed supplement for 5 wk

Item	Corn silage				Hay				ANOVA <i>P</i> -value ¹							
	EL+		EL−		EL+		EL−		SEM	F	L	B	F × L	F × B	L × B	F × L × B
	HO	MO	HO	MO	HO	MO	HO	MO								
Carotenoid																
Lutein	0.05 ^b	0.04 ^b	0.01 ^b	0.04 ^b	0.12 ^{ab}	0.20 ^a	0.10 ^{ab}	0.12 ^{ab}	0.04	<0.001	0.084	0.148	0.364	0.338	0.657	0.183
Zeaxanthin	0.05	0.02	0.01	0.03	0.10	0.15	0.09	0.11	0.07	0.061	0.477	0.631	0.866	0.550	0.825	0.468
β-Cryptoxanthin	0.15	0.13	0.08	0.11	0.11	0.12	0.09	0.09	0.03	0.328	0.026	0.370	0.157	0.641	0.278	0.378
9-Z-β-carotene	0.13	0.17	0.07	0.14	0.08	0.17	0.06	0.21	0.10	0.922	0.694	0.040	0.462	0.454	0.560	0.908
13-Z-β-carotene	0.22	0.23	0.16	0.20	0.26	0.36	0.20	0.38	0.08	0.012	0.373	0.030	0.736	0.107	0.460	0.715
All-E-β-carotene	1.65 ^{ab}	1.71 ^{ab}	1.21 ^b	1.25 ^{ab}	3.18 ^{ab}	3.64 ^a	2.22 ^{ab}	3.72 ^a	0.83	<0.001	0.209	0.147	0.988	0.206	0.471	0.449
Xanthophyll sum	0.24 ^{bc}	0.18 ^{bc}	0.10 ^c	0.18 ^{bc}	0.34 ^{ab}	0.49 ^a	0.29 ^{bc}	0.34 ^{ab}	0.06	<0.001	0.006	0.061	0.683	0.095	0.685	0.038
Carotene sum	2.00 ^{ab}	2.07 ^{ab}	1.48 ^b	1.62 ^{ab}	3.50 ^{ab}	4.18 ^a	2.46 ^{ab}	4.34 ^a	0.93	<0.001	0.242	0.087	0.950	0.159	0.243	0.478
Carotenoid sum	2.25 ^{ab}	2.25 ^{ab}	1.58 ^b	1.80 ^{ab}	3.83 ^{ab}	4.67 ^a	2.75 ^{ab}	4.68 ^a	0.97	<0.001	0.190	0.080	0.973	0.144	0.428	0.591
Vitamin A																
Retinol	0.89	0.90	0.79	1.05	0.90	0.57	0.89	0.64	0.31	0.242	0.841	0.540	0.999	0.120	0.536	0.751
Vitamin A activity ²	3.56	3.64	3.09	4.01	3.97	3.02	3.62	3.28	1.11	0.820	0.917	0.870	0.999	0.231	0.436	0.902
Tocopherol																
α-Tocopherol	5.51	5.23	5.15	5.50	9.39	6.31	6.42	5.31	1.54	0.037	0.146	0.134	0.157	0.121	0.345	0.613
γ-Tocopherol	1.49	1.22	1.18	1.11	1.73	1.17	1.27	1.12	0.24	0.507	0.043	0.023	0.847	0.373	0.177	0.615
Tocopherol sum	7.65	6.66	6.02	6.29	10.93	7.68	7.64	6.34	1.85	0.078	0.044	0.101	0.399	0.234	0.336	0.825

^{a-c}Means (*n* = 3) within a row not sharing a common superscript differ (*P* < 0.05) due to interactions between forage nature, lipid supplement, or cow breed, or all 3.
¹Probability of significant effects due to the nature of the forage (F), the presence (EL+) or not (EL−) of extruded linseed in the diet (L), the breed of the cows (B), and their interactions (F × L, F × B, L × B, and F × L × B).
²The vitamin A activity (expressed in IU/L) includes the respective vitamin A activities from retinol, β-carotene, and β-cryptoxanthin.

Table 6. Milk concentrations (mg/kg of fat) of carotenoids, vitamin A, and vitamin E of Holstein (HO) or Montbéliarde (MO) cows fed diets based on hay or corn silage with or without extruded linseed supplement for 5 wk

Item	Corn silage						Hay						ANOVA <i>P</i> -value ¹						
	EL+			EL−			EL+			EL−									
	HO	MO	SEM	HO	MO	SEM	HO	MO	SEM	HO	MO	SEM	F	L	B	F × L	F × B	L × B	F × L × B
Carotenoid																			
Lutein	0.87	0.71	0.18	1.18	1.16	1.62	1.59	1.58	0.71	0.026	0.889	0.285	0.611	0.769	0.564	0.180			
Zeaxanthin	0.01	0.02	0.01	0.44	0.35	0.62	0.26	0.18	0.34	0.153	0.840	0.271	0.113	0.665	0.877	0.183			
β-Cryptoxanthin	0.03 ^b	0.15 ^{ab}	0.07 ^{ab}	0.16 ^{ab}	0.03 ^b	0.16 ^{ab}	0.02 ^b	0.29 ^a	0.08	0.570	0.228	<0.001	0.653	0.175	0.468	0.227			
13-Z-β-carotene	0.09	0.43	0.17	0.12	0.13	0.29	0.07	0.27	0.14	0.833	0.229	0.012	0.560	0.827	0.144	0.078			
All-E-β-carotene	3.78	3.99	4.22	5.21	5.67	4.89	7.01	5.74	1.69	0.044	0.209	0.764	0.858	0.275	0.915	0.659			
Xanthophyll sum	0.89	1.07	0.22	1.61	1.52	2.19	1.92	2.22	0.96	0.032	0.861	0.127	0.721	0.736	0.600	0.334			
Carotene sum	3.85	4.43	4.38	5.34	5.82	5.14	7.09	6.04	1.73	0.048	0.244	0.945	0.804	0.283	0.997	0.798			
Carotenoid sum	4.78	4.94	4.87	6.81	7.24	8.00	8.82	8.29	1.98	0.005	0.294	0.483	0.981	0.575	0.884	0.358			
Vitamin A																			
Retinol	6.59	8.42	7.69	5.79	5.28	4.12	6.69	6.63	2.27	0.142	0.574	0.734	0.170	0.781	0.547	0.212			
Vitamin A activity ²	22.0	28.1	25.7	19.3	17.6	13.7	22.3	22.1	7.6	0.144	0.570	0.734	0.170	0.778	0.550	0.213			
Tocopherol																			
α-Tocopherol	15.9	14.5	14.7	12.2	16.4	13.8	18.2	16.5	2.5	0.084	0.812	0.067	0.095	0.924	0.960	0.664			
γ-Tocopherol	10.6	8.7	10.3	7.9	9.1	14.6	8.7	13.9	3.4	0.146	0.727	0.271	0.996	0.021	0.907	0.985			
Tocopherol sum	26.1	23.3	24.6	20.0	26.6	28.1	26.5	30.7	5.3	0.058	0.807	0.846	0.455	0.158	0.932	0.613			

^{a,b}Means (*n* = 3) within a row not sharing a common superscript differ (*P* < 0.05) due to interactions between forage nature, lipid supplement, or cow breed, or all 3.¹Probability of significant effects due to the nature of the forage (F), the presence (EL+) or not (EL−) of extruded linseed in the diet (L), the breed of the cows (B), and their interactions (F × L, F × B, L × B, and F × L × B).²The vitamin A activity (expressed in IU/kg of fat) includes the respective vitamin A activities from retinol, β-carotene, and β-cryptoxanthin.

Table 7. Apparent transfer rate (%) of carotenoids, vitamin A, and vitamin E from diet to milk in Holstein (HO) or Montbéliarde (MO) cows fed diets based on hay or corn silage with or without extruded linseed supplement for 5 wk

Item	Corn silage						Hay						ANOVA <i>P</i> -value ¹							
	EL+			EL−			EL+			EL−			SEM	F	L	B	F × L	F × B	L × B	F × L × B
	HO	MO		HO	MO		HO	MO		HO	MO									
Carotenoid																				
Lutein	0.24	0.12	0.06	0.24	0.16	0.16	0.11	0.16	0.17	0.16	0.12	0.12	0.734	0.985	0.577	0.518	0.958	0.223	0.076	
Zeaxanthin	0.01	0.01	0.00	0.32	0.32	0.14	0.14	0.29	0.11	0.08	0.19	0.19	0.408	0.857	0.170	0.096	0.521	0.663	0.126	
13-Z-β-carotene	0.32 ^b	1.79 ^a	0.76 ^{ab}	0.52 ^{ab}	0.86 ^{ab}	0.40 ^{ab}	0.40 ^{ab}	0.86 ^{ab}	0.19 ^b	0.64 ^{ab}	0.51	0.51	0.154	0.184	0.021	0.667	0.722	0.065	0.068	
All-E-β-carotene	2.46	1.80	2.49	2.47	1.89	0.34	0.34	1.89	3.05	2.02	0.79	0.79	0.960	0.277	0.117	0.921	0.569	0.956	0.364	
Xanthophyll sum	0.17	0.13	0.05	0.24	0.15	0.10	0.10	0.15	0.13	0.15	0.10	0.10	0.774	0.916	0.229	0.779	0.674	0.275	0.127	
Carotene sum	2.05	1.61	2.11	2.05	1.52	1.84	1.84	1.52	2.38	1.64	0.62	0.62	0.672	0.290	0.144	0.882	0.614	0.958	0.441	
Carotenoid sum	0.72	0.51	0.64	0.73	0.40	0.37	0.37	0.40	0.54	0.42	0.14	0.14	0.002	0.209	0.322	0.915	0.928	0.464	0.077	
Vitamin A																				
Vitamin A activity ²	0.025	0.019	0.027	0.013	0.014	0.018	0.018	0.014	0.020	0.016	0.006	0.006	0.154	0.945	0.017	0.423	0.385	0.567	0.469	
Tocopherol																				
α-Tocopherol	1.51 ^{ab}	1.03 ^b	1.31 ^{ab}	0.94 ^b	1.22 ^{ab}	1.65 ^{ab}	1.65 ^{ab}	1.22 ^{ab}	1.98 ^a	1.54 ^{ab}	0.26	0.26	0.002	0.466	0.001	0.056	0.972	0.814	0.781	
γ-Tocopherol	1.54	0.97	1.45	1.01	2.05	1.53	1.53	2.05	1.66	2.27	0.52	0.52	0.011	0.728	0.881	0.645	0.031	0.804	0.980	
Tocopherol sum	1.47	1.02	1.35	0.96	1.49	1.67	1.67	1.49	1.85	1.84	0.31	0.31	0.001	0.548	0.059	0.209	0.255	0.671	0.855	

^{a,b}Means (n = 3) within a row not sharing a common superscript differ (*P* < 0.05) due to interactions between forage nature, lipid supplement, or cow breed, or all 3.

¹Probability of significant effects due to the nature of the forage (F), the presence (EL+) or not (EL-) of extruded linseed in the diet (L), the breed of the cows (B), and their interactions (F × L, F × B, L × B, and F × L × B).

²The vitamin A activity includes the respective vitamin A activities from retinol, β-carotene, and β-cryptoxanthin.

but was higher in HO than in MO cows (+44%, $P = 0.017$, Table 7). The general trend for vitamins E was that transfer rates were higher when cows were fed hay alone than when fed corn silage: 1.60 versus 1.20% for α -tocopherol ($P = 0.002$), 1.88 versus 1.24% for γ -tocopherol ($P = 0.011$), and 1.71 versus 1.20% for tocopherol sum ($P = 0.001$). However, for γ -tocopherol, the effect of forage nature was only significant for MO cows ($F \times B$, $P = 0.031$). Moreover, for α -tocopherol, it was more significant when cows did not received EL ($F \times L$, $P = 0.056$). Finally, our data show that α -tocopherol transfer rate was higher in HO than in MO cows (+36%, $P = 0.001$).

DISCUSSION

Carotenoid, Tocopherol, and Vitamin A Status in Cows Fed Different Forages

One of the goals of the experimental design was to compare the status and apparent transfer rate of carotenoids, tocopherols, and vitamin A activity between diet and cow milk in different dietary conditions. As expected, the average amounts of carotenoids ingested per cow per day were higher when cows were fed hay dried in barn than when fed corn silage. On the other hand, the α -tocopherol intake was lower in the former dietary situation. Nevertheless, both carotenoid and α -tocopherol plasma concentrations were higher in cows fed the hay diet. For carotenoids, this observation is in agreement with the dose-response relationship between intake and plasma concentration observed by Calderon et al. (2007) and suggests that the source of these compounds, in term of feedstuff sort, is not very important regardless of their amount. In contrast, the equivalent dose-response relationship for α -tocopherol that seemed clear in experimental conditions where the diet composition was almost the same between treatments (Calderon et al., 2007) would depend on the source of α -tocopherol (bioaccessibility) or the diet composition (lipid content; Reboul, 2017). Literature suggests that vitamin E is not degraded by rumen microorganisms, whatever the diet (Leedle et al., 1993; Weiss et al., 1995). The present results showing higher plasma α -tocopherol concentrations when cows ingested lower amounts with hay would be in agreement with studies performed in humans, suggesting that an intrinsic biochemical factor of the vegetal matrix would modulate α -tocopherol bioaccessibility. However, to date, the identification of this factor remains a failure even though specific studies were performed to explore the interferences linked to pectin, cell walls, or condensed tannins in the α -tocopherol bioaccessibility between vegetal feedstuffs (Sriwichai et al., 2016; Reboul,

2017). Literature also indicates that increasing vitamin A levels in the diet of lactating cows led to a strong decrease in plasma α -tocopherol concentrations (Schelling et al., 1995; Reboul, 2017) but vitamin A intakes were marginally different between experimental groups in the present work, resulting only in the difference in pro-vitamin A carotenoids. Elsewhere, because they are using common uptake pathways (protein transporters SR-B1, NCP1L1, and CD36), competition could occur for absorption between α -tocopherol, carotenoids, vitamins D and K, and γ -tocopherol (Reboul, 2017). It is unlikely that it could be the explanation for the present observations because carotenoid intake was higher (and vitamin D probably also) when cows were fed hay, whereas γ -tocopherol intake was not significantly different. However, the hypothesis remains to be explored for vitamin K, as well as other biological factors associated with the diet.

As a result of higher plasma carotenoid concentrations, of the milk fat yield and in spite of the higher milk yield that had a diluting effect, the milk carotenoid concentrations were higher in cows fed hay, in agreement with previous reports (review in Nozière et al., 2006). Similarly, α -tocopherol concentration in milk fat was higher when cows were fed hay, in agreement with their higher plasma concentration, as already demonstrated by Calderon et al. (2007), although these authors observed lower values. In contrast, milk vitamin A concentration (in $\mu\text{g/mL}$) was lower when cows were fed hay, likely because of the diluting effect caused by the higher milk yield, because the difference disappeared when concentrations were related to milk fat. The daily secretion in milk was higher for cows fed hay than for cows fed corn silage for the main carotenoids and for tocopherols, but lower for retinol (Supplemental Table S2; <https://doi.org/10.3168/jds.2018-16138>). However, the apparent transfer rate from diet to milk was significantly lower for carotenoids but higher for α -tocopherol in cows fed hay in comparison to cows fed corn silage. To our knowledge, such results are not available in the literature. First, it is worth mentioning that transfer rate levels are very low for total vitamin A (around 0.02%, as a result of the dietary vitamin complement) and xanthophylls (lower than 0.2%) and remain low for tocopherols (1 to 2%) and all-E- β -carotene (around 2.3%). Second, for carotenoids, the results confirmed that the process is quantitatively limited because in spite of higher dietary intake with hay, the increase in their milk secretion is limited. It could result simultaneously from limited accessibility of the carotenoids from the vegetal matrix (Sriwichai et al., 2016), digestibility that was demonstrated to be dose dependent and saturable (Nozière et al., 2006), and transfer from plasma to milk (Knowles et al., 2006). In-

deed, according to these latter authors, the calculation of a milk:plasma concentration ratio gives values lower than 0.1% for all-E- β -carotene, around 0.1 and 0.27% for α -tocopherol and retinol, respectively. This would indicate a major physiological barrier at the level of the mammary gland [limited uptake from blood or binding to β -LG (or both), or saturation of milk fat globules; Alvarez et al., 2015].

Effect of Dietary Lipid Supplementation on Carotenoid, Tocopherol, and Vitamin A Status

The main hypothesis in the present work was based on the digestive and metabolic links between carotenoids, vitamin A, and vitamin E on the one hand, and lipids on the other hand. We aimed to improve the general status (bioavailability and plasma concentrations) of the dairy cows and increase the efficiency of carotenoid and tocopherol transfer from diet to milk, through a dietary fat supplement. We choose EL, in line with their more and more frequent utilization in the diet formulation of ruminants. As demonstrated here, EL does not contain carotenoids but tocopherols (two-thirds γ and one-third α). Thus, when cows received EL in the diet, the tocopherol intake increased. Moreover, the general trend was also an increase in carotenoid intake in the cows fed hay and not when fed corn silage. Indeed, for the hay-based diet, the inclusion of EL led to a decrease in distribution of soybean meal and cereal mix but an increase in the proportion of hay.

However, whatever the forage composition, EL supplementation numerically increased the plasma carotenoid concentrations, reaching significance for xanthophyll sum. This effect not being limited to cows fed hay would indicate that EL supplementation improved carotenoid status of cows, possibly through an increase in dietary carotenoid accessibility. Additionally, EL supplementation induced a significant increase in plasma tocopherol concentration. To our knowledge, this is the first report of these observations. Indeed, when Puppel et al. (2016) studied the effect of an n-3-rich supplement on antioxidants in dairy cows, they did not make measurements at the plasma level. In contrast, when dairy cows received a corn-silage-based diet supplemented by EL at 50 g/kg of DM, plasma α -tocopherol concentration was not different in comparison to the control group and γ -tocopherol concentration was not determined (Gobert et al., 2009). In the present work also, α -tocopherol concentration was not statistically significant (but numerically) between groups receiving EL or not, but γ -tocopherol concentration and the sum were. It could result, at least in part, to their respective intakes in agreement with the dose-response observed by Calderon et al. (2007).

Consequences of EL in the diet were the improvement of the milk, fat, and protein yields, but as the increase in milk yield was higher, milk fat and protein content were lower. Resulting from a diluting effect, carotenoid concentrations in milk were lower with EL in the diets. However, this effect disappeared when concentrations were related to milk fat. Moreover, EL addition in the diet did not modify the amounts of carotenoid secreted per day as well as their apparent transfer rate from diet to milk. Thus, it would seem that EL addition in the diet could improve carotenoid bioavailability in the dairy cows fed either hay or corn silage, as illustrated by their plasma concentration, but not the transfer from plasma to milk, in contrast to previous observation (Puppel et al., 2016). Indeed, the milk:plasma ratio was lower for carotenoids when cows ate EL than when they did not (0.068 vs. 0.103, respectively). Thus, even though increasing the milk β -carotene concentration through dietary lipid supplementation seems possible (Puppel et al., 2016), it was not observed in our experimental conditions. However, in Puppel et al. (2016), the lipid supplementation induced an increase in milk fat content whereas the opposite was observed in the present experiment, suggesting different effects on the lipid metabolism of the dairy cows between studies. The discrepancies could be linked to differences in the cow breeds (Polish Holstein-Friesian vs. HO and MO), the average lactation stages at the beginning of the experiment (170 vs. 66 DIM), the average milk yields (21.1 vs. 31.3 kg/d), or a combination of these. The source of fat was also different between the 2 studies (fish oil and unprocessed whole linseeds vs. EL) but the doses seemed similar. Moreover, it could also possibly result from the level of carotenoid ingested by cows that should be higher in Puppel et al. (2016) because of the high proportion of alfalfa silage in the diet (Nozière et al., 2006; Calderon et al., 2007). The comparison of milk β -carotene concentration supports this hypothesis because values were between 0.45 and 1.1 mg/L in Puppel et al. (2016) and 0.16 to 0.22 mg/L (Supplemental Table S1; <https://doi.org/10.3168/jds.2018-16138>) in the present study. It could suggest that dietary lipids become limiting for the efficient carotenoid absorption only when carotenoid intake levels are relatively high.

In the present study, the effects of EL on milk α -tocopherol were dependent on the nature of the forage in the diet, highlighting the interest in the experimental design. Indeed, when fed hay with EL, cows produced a milk poorer in α -tocopherol than when fed hay without EL (whether the concentration is reported to volume or to milk fat), secreted less α -tocopherol in the milk per day, and the efficiency of its transfer from diet to milk was also reduced. In contrast, when fed corn silage as forage, cows produced a milk fat richer in α -tocopherol,

secreted more α -tocopherol in the milk per day, and the efficiency of its transfer from diet to milk was increased. Puppel et al. (2016) also observed that the dietary fat supplement induced an increase in milk α -tocopherol concentration simultaneously to the increase in milk yield, indicating a rise in the α -tocopherol daily secretion. However, as in the present experiment, dietary lipid supplements given in a diet rich in starch often induced a fat milk depression in cows, particularly when lipids are of plant origin (Bernard et al., 2018). This effect is mediated by *trans*-10,*cis*-12 CLA originating from rumen biohydrogenations of dietary long-chain fatty acids. The CLA isomer possesses an antilipogenic effect through the downregulation of the expression of genes implied in de novo lipogenesis, lipid uptake, and synthesis in the cow mammary gland (Bernard et al., 2018). Gessner et al. (2015) explored the effect of CLA on retinol and tocopherol status in early lactation dairy cows. They observed that plasma concentrations were not different between CLA-supplemented or nonsupplemented cows; retinol and tocopherol concentrations in milk as well as their daily secretion (in spite of the induced milk fat depression) increased when cows received the CLA supplement. In the present study, the pattern of response was different since EL supplement increased tocopherol plasma concentration whatever the forage, but reduced tocopherol milk concentration, secretion, and apparent transfer rate when cows were fed hay, whereas it increased these parameters when cows were fed corn silage. When fed hay, the milk:plasma ratio was reduced with EL supplement from 0.10 to 0.06, suggesting a lower efficiency of tocopherol transfer across the mammary gland, for an unknown reason (such as downregulation of lipoprotein lipase activity or of low-density lipoprotein receptor). In contrast, when fed corn silage, cows had a milk:plasma ratio maintained at the same level whether they received EL supplement or not (0.10–0.11), suggesting that the EL supplementation that improved the general tocopherol status of the cows and the apparent transfer rate would be a consequence of an increase in dietary tocopherol bioavailability (Borel et al., 2013).

Differences Between Breeds on Carotenoid, Tocopherol, and Vitamin A Status

As expected, HO cows ate more and produced more milk than MO cows. Consequently, HO cows ingested more carotenoids and tocopherols than MO cows. But surprisingly, observations in plasma highlighted higher carotenoid concentrations in MO than HO cows. It is the first time a significant difference was observed for plasma carotenoid concentrations between these 2 breeds, whereas several comparisons have been already

reported by Nozière et al. (2006), always indicating similar mean values. This is especially surprising because MO cows ingested fewer carotenes than HO cows in the present study. It could suggest breed-related differences at the level of carotene digestibility efficiency, conversion to vitamin A, or plasma metabolism/clearance. All these elements being under protein factor dependency (transporters, enzymes, or both), genetic variations are likely to occur, as supported by the fact that plasma carotene concentrations are under the inheritance of the sire (Jensen et al., 1999). When considering the differences between breeds at the milk level, results indicated that HO cows secreted more carotenoids into milk per day even if not significant (+1.43 mg/d on average), which is in line with the higher intake in these cows (+181 mg/d per cow). The numerically lowest concentrations in milk and milk fat in HO cows was likely a consequence of a dilution effect of the milk yield increase in these cows. However, the higher mammary secretion of carotenoids in HO cows could also induce a reduction in their plasma concentration through a clearing effect, but additional studies are required to test this hypothesis.

The effects of breed on vitamin A status are more limited since the difference observed at the level of dietary intake was due to pro-vitamin A carotenoids. However, the amount of retinol secreted in milk per day and the apparent transfer rate of vitamin A activity were significantly higher in HO than in MO cows (+1.53 mg/d and +44%, respectively), whereas concentrations were very close in plasma and in milk, whatever the unit, in agreement with Nozière et al. (2006) and Krol et al. (2017). It suggests that, in our experimental conditions, mammary retinol secretion was driven mainly by milk yield and was not dependent on vitamin A intake (as carotenoids of preformed vitamin A).

Alpha-tocopherol status was completely dependent on the breed since HO cows had higher intake than MO cows for a given diet, had higher plasma and milk concentrations, secreted more α -tocopherol per day, and presented a higher transfer rate. To our knowledge, it is the first time a direct comparison of vitamin E status (intake, plasma concentration, milk secretion, and apparent transfer rate) was performed between these 2 breeds. However, several discrepancies in milk concentrations between breeds have already been reported between Modicana and Brown Swiss or HO (Marino et al., 2012), Jersey and HO cows (Sol Morales et al., 2000), or Danish-Holstein-Friesian, Danish Jersey, and Swedish Red (Poulsen et al., 2012). In these latter studies, authors usually presented milk (and sometimes plasma) concentrations but did not determine vitamin E intake by the cows. In the present study, we observed that milk concentration can differ between breeds, and

it could result from different intake, but not only this because the apparent transfer rate was also different, indicating that for the same intake, the efficiency of α -tocopherol transfer from diet to milk is higher in HO than in MO cows receiving exactly the same diet.

According to breed, the status of the cows regarding γ -tocopherol was different from that of α -tocopherol because the breed effect was largely dependent on the diet fed to cows. Indeed, γ -tocopherol intake was the highest for HO cows fed corn silage and the lowest for MO cows fed hay. However, for milk concentrations, amount secreted daily by the udder, and apparent transfer rate from diet to milk, MO cows fed hay gave the highest values, whereas the lowest values were obtained from MO cows fed corn silage, with HO cows having intermediate values. Finally, plasma concentrations highlighted higher values for HO than MO cows, whatever the diet. Differences between breeds are original, but Gessner et al. (2015) proved, through CLA dietary supplementation, that γ -tocopherol secretion in milk is under the control of regulatory processes. Here, we describe that forage nature of the diet and cow breed, but not plasma γ -tocopherol concentration, could regulate γ -tocopherol secretion in milk.

CONCLUSIONS

The present work highlighted for the first time the differences in carotenoid and vitamin A and E status in term of intakes, plasma and milk concentrations, and efficiency of apparent transfer rates from diet to milk between cows from 2 breeds (HO and MO) fed 2 distinct diets that mainly differed according to forage composition. Whereas hay feeding corresponded to superior intake and animal status in carotenoids, the apparent transfer rate was lower than when cows were fed corn silage, indicating a limited process. In contrast, even though tocopherol intakes were lower when fed hay, the animal status was unexpectedly and for an unknown reason higher than when cows were fed corn silage. As expected, the dietary EL supplementation increased the availability of carotenoids and tocopherols likely by improving their digestibility as indicated by their higher plasma concentrations. However, lipid supplementation did not have an effect on milk carotenoid secretion and a variable effect on tocopherol secretion according to forage composition, in line with the development of a milk-fat depression when cows received a starch-rich diet. The mammary gland transfer seems a key limiting step to overcome for milk lipid enrichment with these compounds. This is a reason why additional studies would be required to understand better the regulations in uptake, intracellular transport, and secretion in milk at this level.

ACKNOWLEDGMENTS

The authors thank O. Troquier, D. Baguet, and the team of the Herbipole experimental unit of Marcenat (INRA, Marcenat, France) for the feeding, milking, and diligent management of experimental cows, B. Chauveau-Duriot and I. Kerveillant (INRA, Saint-Genès Champanelle, France) for technical support during the analysis, and L. Bernard (INRA, Saint-Genès Champanelle, France) for helpful discussions on milk-fat depression mechanisms. This research was supported in part by the European TRUEFOOD programme (Traditional United Europe Food), an integrated project financed by the European Commission under the 6th Framework Programme for RTD (contract no. FOOD-CT-2006-016264), and by the national French PNRA Agilait programme (structures, oxidation stability, properties, and bioaccessibility of the milk fat of dairy products enriched in unsaturated fatty acids; project no. ANR-06-PNRA-012). This work was performed thanks to the program “Investissement d’Avenir” (16-IDEX-0001 CAP 20-25) funded by the Agence Nationale de la Recherche of the French government. Authors declare no conflict of interest.

REFERENCES

- Agabriel, C., A. Cornu, C. Journal, C. Sibra, P. Grolier, and B. Martin. 2007. Tanker milk variability according to farm feeding practices: Vitamins A and E, carotenoids, color, and terpenoids. *J. Dairy Sci.* 90:4884–4896.
- Alvarez, R., A. J. Melendez-Martinez, I. M. Vicario, and M. J. Alcázar. 2015. Carotenoid and vitamin A contents in biological fluids and tissues of animals as an effect of the diet: A review. *Food Rev. Int.* 31:319–340.
- AOAC International. 1997. Official Methods of Analysis, 16th ed. AOAC International, Gaithersburg, MD.
- Bernard, L., M. Bonnet, C. Delavaud, M. Delosière, A. Ferlay, H. Fougère, and B. Graulet. 2018. Milk fat globule in ruminant: Major and minor compounds, nutritional regulation and differences among species. *Eur. J. Lipid Sci. Technol.* 120:1700039.
- Borel, P., D. Prevaud, and C. Desmarchelier. 2013. Bioavailability of vitamin E in humans: An update. *Nutr. Rev.* 71:319–331.
- Brown, M. J., M. G. Ferruzzi, M. L. Nguyen, D. A. Cooper, A. L. Eldridge, S. J. Schwartz, and W. S. White. 2004. Carotenoid bioavailability is higher from salads ingested with full-fat than with fat-reduced salad dressings as measured with electrochemical detection. *Am. J. Clin. Nutr.* 80:396–403.
- Calderon, F., B. Chauveau-Duriot, P. Pradel, B. Martin, B. Graulet, M. Doreau, and P. Nozière. 2007. Variations in carotenoids, vitamins A and E, and color in cow's plasma and milk following a shift from hay diet to diets containing increasing levels of carotenoids and vitamin E. *J. Dairy Sci.* 90:5651–5664.
- Chauveau-Duriot, B., M. Doreau, P. Nozière, and B. Graulet. 2010. Simultaneous quantification of carotenoids, retinol, and tocopherols in forages, bovine plasma, and milk: Validation of a novel UPLC method. *Anal. Bioanal. Chem.* 397:777–790.
- Crisa, A. 2013. Milk carbohydrates and oligosaccharides. Pages 129–147 in *Milk and Dairy Products in Human Nutrition: Production, Composition and Health*. Y. W. Park and G. F. W. Haenlein, ed. Wiley-Blackwell, Chichester, UK.
- Gaucheron, F. 2013. Milk minerals, trace elements, and macroelements. Pages 172–199 in *Milk and Dairy Products in Human Nu-*

- trition: Production, Composition and Health. Y. W. Park and G. F. W. Haenlein, ed. Wiley-Blackwell, Chichester, UK.
- Gessner, D. K., E. Most, G. Schlegel, K. Kupczyk, F. J. Schwarz, and K. Eder. 2015. Concentrations of retinol and tocopherols in the milk of cows supplemented with conjugated linoleic acid. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 99:1039–1046.
- Gobert, M., B. Martin, A. Ferlay, Y. Chilliard, B. Graulet, P. Pradel, D. Bauchart, and D. Durand. 2009. Plant polyphenols associated with vitamin E can reduce plasma lipoperoxidation in dairy cows given n-3 polyunsaturated fatty acids. *J. Dairy Sci.* 92:6095–6104.
- Gordon, M. H. 2013. Milk lipids. Pages 65–79 in *Milk and Dairy Products in Human Nutrition: Production, Composition and Health*. Y. W. Park and G. F. W. Haenlein, ed. Wiley-Blackwell, Chichester, UK.
- Graulet, B., B. Martin, C. Agabriel, and C. L. Girard. 2013. Vitamins in milk. Pages 200–219 in *Milk and Dairy Products in Human Nutrition: Production, Composition and Health*. Y. W. Park and G. F. W. Haenlein, ed. Wiley-Blackwell, Chichester, UK.
- INRA (Institut National de la Recherche Agronomique). 2007. *Alimentation des bovins, ovins et caprins. Besoins des Animaux – Valeur des aliments – Tables INRA 2007*. Editions Quae, Versailles, France.
- Jensen, S. K., A. K. Bjornbak Johannsen, and J. E. Hermansen. 1999. Quantitative secretion and maximal secretion capacity of retinol, β -carotene and α -tocopherol into cows' milk. *J. Dairy Res.* 66:511–522.
- Knowles, S. O., N. D. Grace, T. W. Knight, W. C. McNabb, and J. Lee. 2006. Reasons and means for manipulating the micronutrient composition of milk from grazing dairy cattle. *Anim. Feed Sci. Technol.* 131:154–167.
- Korhonen, A. J., and P. Marnila. 2013. Milk bioactive proteins and peptides. Pages 148–171 in *Milk and Dairy Products in Human Nutrition: Production, Composition and Health*. Y. W. Park and G. F. W. Haenlein, ed. Wiley-Blackwell, Chichester, UK.
- Krol, J., A. Brodziak, A. Zaborska, and Z. Litwinczuk. 2017. Comparison of whey proteins and lipophilic vitamins between four cow breeds maintained in intensive production system. *Mljekarstvo* 67:17–24.
- Kukovics, S., and T. Németh. 2013. Milk major and minor proteins, polymorphisms and non-protein nitrogen. Pages 80–110 in *Milk and Dairy Products in Human Nutrition: Production, Composition and Health*. Y. W. Park and G. F. W. Haenlein, ed. Wiley-Blackwell, Chichester, UK.
- Leedle, R. A., J. A. Z. Leedle, and M. D. Butine. 1993. Vitamin-E is not degraded by ruminal microorganisms - Assessment with ruminal contents from a steer fed a high-concentrate diet. *J. Anim. Sci.* 71:3442–3450.
- Marino, V. M., I. Schadt, S. La Terra, M. Manenti, M. Caccamo, G. Licitra, and S. Carpino. 2012. Influence of season and pasture feeding on the content of alpha-tocopherol and beta-carotene in milk from Holstein, Brown Swiss and Modicana cows in Sicily. *Dairy Sci. Technol.* 92:501–513.
- Nozière, P., B. Graulet, A. Lucas, B. Martin, P. Grolier, and M. Doreau. 2006. Carotenoids for ruminants: From forages to dairy products. *Anim. Feed Sci. Technol.* 131:418–450.
- Poulsen, N. A., F. Gustavsson, M. Glantz, M. Paulsson, L. B. Larsen, and M. K. Larsen. 2012. The influence of feed and herd on fatty acid composition in 3 dairy breeds (Danish Holstein, Danish Jersey, and Swedish Red). *J. Dairy Sci.* 95:6362–6371.
- Puppel, K., B. Kuczyńska, T. Nalecz-Tarwacka, M. Golebiewski, T. Sakowski, A. Kapusta, A. Budzinski, and M. Balcerak. 2016. Effect of supplementation of cows diet with linseed and fish oil and different variants of beta-lactoglobulin on fatty acid composition and antioxidant capacity of milk. *J. Sci. Food Agric.* 96:2240–2248.
- Reboul, E. 2017. Vitamin E bioavailability: Mechanisms of intestinal absorption in the spotlight. *Antioxidants* 6:95. <https://doi.org/10.3390/antiox6040095>.
- Schelling, G. T., R. A. Roeder, M. J. Garber, and W. M. Pumfrey. 1995. Bioavailability and interaction of vitamin-A and vitamin-E in ruminants. *J. Nutr.* 125:1799S–1803S.
- Sol Morales, M., D. L. Palmquist, and W. P. Weiss. 2000. Milk fat composition of Holstein and Jersey cows with control or depleted copper status and fed whole soybeans or tallow. *J. Dairy Sci.* 83:2112–2119.
- Sriwichai, W., J. Berger, C. Picq, and S. Avallone. 2016. Determining factors of lipophilic micronutrient bioaccessibility in several leafy vegetables. *J. Agric. Food Chem.* 64:1695–1701.
- Veissier, I. 1999. *Expérimentation animale: Biologie, éthique, réglementation*. INRA Prod. Anim. 12:365–375.
- Weiss, W. P., K. L. Smith, J. S. Hogan, and T. E. Steiner. 1995. Effect of forage to concentrate ratio on disappearance of vitamin-A and vitamin-E during in vitro ruminal fermentation. *J. Dairy Sci.* 78:1837–1842.