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Effects of new n-3 fatty acid sources on milk fatty acid profile and milk fat properties in dairy cows

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Feeding livestock with n-3 fatty acid (FA) sources (linseed, for example) is a common strategy to improve lipid quality of meat and milk products. However, in monogastric animals, linseed tegument decreases digestibility and alphalinolenic acid (ALA) uptake, while the whole linseed is well used by ruminants. In a context of increasing sustainability of feeding systems, providing monogastric animals and ruminants with linseed products adapted to their digestive systems is an important issue. This research paper addresses the hypotheses: (i) sieved extruded linseed (SEL) specific for ruminants is as or more effective than standard extruded linseed (ii) microalgae DHA Gold® is an interesting source of docosahexaenoic acid (DHA) in feedstuff and (iii) the effects of SEL and microalgae on milk characteristics are complementary and additive. Thirty-two cows were divided into 4 groups with different dietary n-3 fatty acid sources using a continuous design. All the diets were fed as mixed rations based on maize silage, energy concentrate and soybean meal. The first group received a control diet (CTRL) with no additional fat. The 3 other groups received SEL, microalgae DHA Gold® (ALG) and a mixture of microalgae DHA Gold® and SEL (SEL/ALG). Milk was collected from morning milkings after six weeks of dietary treatment. In SEL and SEL/ALG, ALA increased (+0·32 and +0·26% unit, respectively), and DHA increased in ALG and SEL/ALG (+0·43 and +0·15% unit, respectively) compared to CTRL, as a consequence of the initial composition of the n-3 FA sources. In SEL, milk yield, fat and protein contents, milk fat globule size and spontaneous lipolysis (measured to evaluate suitability for milk processing) were not different compared with CTRL. In ALG and SEL/ALG, milk yield decreased (−2·8 and −6·0 kg/d, respectively), fat content was halved, and fat globule size was reduced (−1·46 and −1·31 µm, respectively) compared to CTRL. Spontaneous lipolysis increased in ALG (+0·12 mEq/kg of milk) compared to CTRL. Protected microalgae and the doses of microalgae in the diet need further investigation to prevent FA modification in the rumen and the consequent deleterious effects on milk fat.

Keywords: Flaxseed, milk quality, microalgae, dairy cow, lipolysis.

Alphalinolenic (ALA) and docosahexaenoic acids (DHA) are essential for the maintenance of normal health and nutrition. However, they cannot be synthesised by the body and must be supplied by the diet (Calder & Yaqoob, 2009). Intake of ALA and DHA is low in Western countries, therefore, increasing the average intake of n-3 FA is a public-health issue. To meet this goal, one strategy is to provide livestock diets supplemented with sources of n-3 polyunsaturated FA (PUFA) to enhance the n-3 FA content of meat, eggs, milk and milk products (Calder & Yaqoob, 2009).

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Linseed, particularly rich in ALA [representing more than 50% of linseed total fatty acid (FA)], has been widely studied and is commonly used in monogastric animals and ruminants diets in different feed forms, i.e., whole seed, micronised, heated or extruded seed, and oil (Gonthier et al. 2005; Noblet et al. 2008). Linseed is composed of a tegument, rich in fibre, mucilage, tannins, and cyanogen compounds which surround the kernel which contains the nutritive reserves of linseed (Kadivar, 2001). In monogastric animals, the tegument decreases linseed digestibility, leading to a poorer absorption of nutrients, including ALA (Noblet et al. 2008), while the whole linseed (tegument plus kernel) is digested and well used by ruminants when linseed is provided under 4% DMI with no impact on ALA

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transfer efficiency from feedstuff to milk (Gonthier et al. 2004; Martin et al. 2008). In a context of increasing sustainability of feeding systems, providing monogastric animals and ruminants with linseed products adapted to their digestive systems is an important issue. By a process of sieving and sifting classically used in flour-milling industry, tegument and kernel of extruded linseed have been separated to be respectively directed to the production of feedstuff specific to ruminants and monogastric animals (Valorex, Combourtillé, France). We hypothesised that valorisation by dairy cows of sieved extruded linseed (SEL) would be as or more effective than standard extruded linseed.

However, linseed, does not contain DHA, the principal natural source of which is seafood (Calder & Yaqoob, 2009). Thus, ingredients such as microalgae have been experimentally tested in monogastric and ruminant diets (AbuGhazaleh et al. 2009; Stamey et al. 2012; Baeza et al. 2013; Bragaglio et al. 2015; De Tonnac et al. 2017). Even combinations between ALA and DHA sources (linseed oil and microalgae) have been studied to improve milk and meat FA profile (Angulo et al. 2012; De Tonnac et al. 2017). In our experiment, DHA Gold®, obtained by drum-drying Schizochytrium algae, which contains a large amount of DHA has been used in dairy cow diets alone and in combination with SEL, specific for ruminants, to improve nutritional profile of milk. We hypothesised that the effects of ALA and DHA sources (SEL and DHA Gold®) on milk would be additive, particularly in relation to milk FA profile. Milk FA composition was measured to evaluate nutritional aspect of the milk. Milk fat globule (MFG) size and spontaneous lipolysis (SL) were also measured to evaluate suitability of milk to processing.

Material & methods

The protocol was approved by an ethics committee for animal experimentation under number 01421·02.

Animals

Thirty-two multiparous ($n = 16$) and primiparous ($n = 16$) Holstein dairy cows in mid-lactation were used. At the beginning of the experiment, the cows were at day 100 ± 17·5 of lactation. During the pre-experimental period, milk yield, milk-solids content, and milk monounsaturated (MUFA) and PUFA percentages were evaluated to allocate the cows into four groups. Criteria for blocking were, in order, milk yield, solids content, parity (primiparous, multiparous), lactation stage, DMI, and milk MUFA and PUFA percentages. Each group was composed of four primiparous and four multiparous cows. Mean values are presented in Supplementary Table S1. All cows were kept indoors with an average area of 6·56 m^2 per cow. Cows were milked at 0700 and 1700 h in a milking parlour. The cows were weighed after milking.

Diet treatments

Four diet treatments were fed as total mixed rations. They were based on maize silage, a variable part of energy concentrate and n-3 FA sources, soybean meal, urea and vitamins. The new extruded linseed (EL) product was obtained by a process of linseed sieving and sifting that induced a separation between particles of different sizes. Fine particles represented the linseed kernel and the coarse particles represented the linseed teguments that were extruded. The first group received a control diet (CTRL) with no additional fat. The 3 other groups received sieved EL (SEL), microalgae DHA Gold® (ALG) and a mixture of microalgae DHA Gold® and SEL (SEL/ALG) (Table 1).

Diets were formulated to meet the energy and protein requirements, based on milk production and milk solid content measured during the pre-experimental period (Institut National de la Recherche Agronomique, 2007). The ingredients, chemical composition and nutritional value of the diets are given in Supplementary Table S2 and in Table 1. The cows were fed ad libitum. The feeds were weighed and mechanically distributed twice daily at 0900 and 1830 h.

Experimental design

The experiment was conducted over a continuous period of 10 weeks. The experiment started with a covariate period of three pre-experimental weeks during which the cows were fed the CTRL diet, which was followed by one week of adaptation to the experimental diets and the six-week experimental period, from 13 January to 23 March 2014. Milk and blood were sampled during the pre-experimental period (covariate period) and during the sixth week of the experimental period.

Feed and refusals

Throughout the experimental period, cows were individually fed *via* individual electronic gating, and all refusals were collected and weighed every day to evaluate daily intake. To determine diet chemical and nutritional composition, samples of fresh maize silage were collected five times a week and samples of energy concentrate, soybean meal, sieved EL, DHA Gold®, DHA Gold®/sieved EL mixture were collected every week throughout the experimental period. The samples were stored at −20 °C and pooled to produce one sample per type of feed and per period. The analyses of the samples are described in Supplementary Material S1.

Milk and fat characteristics

Milk yield was recorded individually every day at each milking. Milk fat, protein and lactose content, and somatic-cell score were determined for four consecutive milkings every week. These analyses were performed by

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Table 1. Composition of experimental diets (CTRL: control, SEL: addition of sieved EL, ALG: addition of DHA Gold® and SEL/ALG: addition of sieved EL and DHA Gold® mixture)

Item	CTRL	SEL	ALG	SEL/ ALG
Ingredients, %DM				
Maize silage	75·7	76·5	75·5	76·1
Energy concentrate†	11·1	5·6	9·2	5·3
Soymeal	10·4	10·0	10·0	9·9
sieved extruded linseed‡	0	5·1	0	0
DHA Gold®§	0	0	1·8	0
DHA Gold®/sieved extruded linseed¶	0	0	0	5·6
Minerals (g)	1·7	1·7	2·0	1·9
Urea (g)	1·1	1·1	1·4	1·2
Chemical composition, g/kg of DM unless noted				
DM, %	45·4	44·3	44·4	42·4
Organic matter	922	923	922	806
CP	151	144	141	154
NDF	338	342	335	368
ADF	180	181	179	185
Starch	241	222	235	222
Fat	32·0	43·9	39·3	35·9
Total n-3 FA	3·14	10·44	7·60	5·23
Monounsaturated FA	7·75	9·33	7·62	9·46
Polyunsaturated FA	18·6	27·6	24·5	19·6
18:2	15·2	16·8	15·0	14·8
18:3, ALA	2·70	9·87	2·68	5·28
20:5, EPA	0·01	0·01	0·09	0·02
22:6, DHA	0·04	0·12	4·25	0·76
Nutritional value, g/kg of DM unless noted				
NE_L, kJ/kg of DM	6·5	6·5	6·5	6·2
PDIE	102	102	98	96
PDIN	106	106	102	98

FA, fatty acid; ALA, alpha linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; NE_L, net energy for lactation; PDIE, protein digested in the small intestine supplied by rumen undegradable protein and by microbial protein from rumen-fermented OM (INRA, 2007); PDIN, protein digested in the small intestine supplied by rumen undegradable protein and by microbial protein from rumen-degraded dietary nitrogen (INRA, 2007).

†Energy concentrate on DM basis: 20% wheat, 20% corn, 20% barley, 20% beet pulp, 15% wheat bran, 3% cane molasses, 1% vegetal oil, 1% salts.

‡sieved extruded linseed = 50% sieved extruded linseed, 50% wheat bran; Valorex, Combourtillé, France.

§DHA Gold®; DSM, Deinze, Belgium.

¶DHA Gold®/sieved extruded linseed = 37% sieved extruded linseed, 50% wheat bran, 13% DHA Gold®; Valorex, Combourtillé, France.

mid-IR spectrometry for fat, protein and lactose content and by flow cytometry for somatic-cell score at the dairy laboratory MyLab (Châteaugiron, France). Milk samples were collected individually from milk cans from one morning milking and one evening milking and pooled at a 60:40 ratio during the pre-experimental period and the last week of the experimental period. For milk FA profile, milk was stored at −20 °C until analysis. The FA composition was determined as described in Hurtaud et al. (2010).

Milk fat globule size was determined as described in Vanbergue et al. (2017). Spontaneous lipolysis was determined as in Vanbergue et al. (2016) from individual milk samples from milk cans collected during the last week of the experimental period during morning milkings.

Calculation and statistical analyses

All statistical analyses were performed using SAS software (SAS 9·2 Institute Inc., Cary, NC). The statistical significance threshold was set to $P < 0{\cdot}05$, and the trend threshold was set to $P < 0{\cdot}10$. The normality of the data was checked using the Shapiro-Wilk test in the SAS-package univariate procedure. The effects of the diets on milk yield, milk composition (except for SL and MFG size), weight, DMI and energy and protein supplies and balances, were analysed using the GLM procedure in SAS according to the following statistical model: $Y_i = \mu + \text{alim}_i + \text{Cov}\ Y_i + \varepsilon_i$, where Y_i is the dependent variable, μ is the mean, alim_i, is the effect of the i diet treatments (CTRL, SEL, ALG, SEL/ALG), Cov Y_i is the covariable associated with Y_i (i.e., the value of Y_i during the pre-experimental period), and ε_i is the residual error. For MFG size, the covariable was the fat content during the pre-experimental period and for SL, there was no covariable. For each model, comparisons were performed with LSMEANS.

Results

Intake and nutrient supply and balance

Total DMI decreased only in ALG and SEL/ALG. As a consequence, intake of net energy for lactation, PDIE (protein digested in the small intestine originating from rumen undegradable protein and by microbial protein from rumen-fermented OM; INRA, 2007) and PDIN (protein digested in the small intestine originating from rumen undegradable protein and by microbial protein from rumen-degraded dietary nitrogen; INRA, 2007) were lower in ALG and SEL/ALG ($P < 0{\cdot}001$). However, net energy and metabolic protein balance stayed positive in SEL/ALG and net energy and PDIN balance stayed positive in ALG. Intake of ALA was higher in SEL and SEL/ALG and lower in ALG than in CTRL ($P < 0{\cdot}001$). Intake of DHA was slightly higher in SEL and was higher in ALG and SEL/ALG than in CTRL ($P < 0{\cdot}001$) (Table 2).

Milk yield and milk protein and fat content

Milk yield, milk fat content, milk fat and protein yield were lower in ALG and SEL/ALG ($P < 0{\cdot}001$). Milk yield was even lower in SEL/ALG. Milk protein content was higher in SEL/ALG compared to the other treatments ($P < 0{\cdot}001$). Lactose content tended to be higher in SEL and ALG than in CTRL and SEL/ALG ($P = 0{\cdot}065$). Somatic-cell score

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Table 2. Effects of addition of sieved EL (SEL), DHA Gold® (ALG) and sieved EL/DHA Gold® mixture (SEL/ALG) on weight, DM, energy and protein intake and balance of dairy cows

	CTRL	SEL	ALG	SEL/ALG	RMSE	Treatment effect
Weight, kg	613	610	593	601	13·62	0·052
Intake, kg of DM/d						
Total	20·8^{a}	19·6^{a}	16·0^{b}	16·9^{b}	1·35	<0·001
As forage	15·8^{a}	15·0^{a}	12·1^{b}	12·8^{b}	1·05	0·0001
As concentrate	5·0^{a}	4·6^{a}	3·9^{b}	4·1^{b}	0·33	<0·001
Intake, g/d unless noted						
NE_L, MJ/d	7·17^{a}	7·03^{a}	5·58^{b}	5·21^{b}	0·347	<0·001
PDIE	2108^{a}	2010^{a}	1574^{b}	1655^{b}	139·6	<0·001
PDIN	2100^{a}	2025^{a}	1665^{b}	1727^{b}	124·4	<0·001
CP	2418^{a}	2280^{a}	1820^{b}	2025^{b}	302·0	0·001
Fat	3246	3171	3250	3393	461·4	0·838
n-3 FA	66·1^{d}	203·9^{a}	135·8^{c}	191·5^{b}	11·57	<0·001
18:2	319·8^{a}	327·1^{a}	238·4^{b}	283·9^{a}	39·36	<0·001
18:3, ALA	56·7^{c}	192·7^{a}	42·9^{d}	145·6^{b}	9·81	<0·001
20:5, EPA	0·23^{c}	0·13^{d}	1·74^{a}	0·81^{b}	0·041	<0·001
22:6, DHA	0·79^{d}	2·3^{c}	81·3^{a}	36·9^{b}	1·40	<0·001
Balance, g/d unless noted						
NE_L, MJ/d	0·58^{b}	0·31^{b}	0·43^{b}	1·11^{a}	0·329	<0·001
PDIE	281^{a}	227^{a}	−76^{c}	36^{b}	107·6	<0·001
PDIN	274^{a}	244^{a}	12^{b}	108^{b}	98·7	<0·001

RMSE, Root mean square error; NE_L, net energy for lactation; PDIE, protein digested in the small intestine supplied by rumen undegradable protein and by microbial protein from rumen-fermented OM (INRA, 2007); PDIN, protein digested in the small intestine supplied by rumen undegradable protein and by microbial protein from rumen-degraded dietary nitrogen (INRA, 2007); ALA, alpha linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.
$^{a–c}$Means in the same row with no common superscript differ ($P < 0·05$).

tended to be higher in ALG and SEL/ALG than in CTRL ($P = 0·063$) (Table 3).

Milk fatty acid profile

The FA profile was affected by the treatments, with the effects being much greater in ALG and SEL/ALG than in SEL. Compared with CTRL, saturated FA (SFA) percentage decreased in the order SEL, ALG, and SEL/ALG ($P < 0·001$), and de novo-synthesised FA percentage (4:0–15:0) was lower in SEL and lower still in ALG and SEL/ALG ($P < 0·001$). The percentage of 16:0 was lower in SEL and SEL/ALG ($P = 0·005$) but was not significantly different between ALG and CTRL. The percentage of 18:0 was higher in SEL than in CTRL, was much lower in ALG and SEL/ALG than in CTRL ($P < 0·001$), and did not differ between SEL/ALG and ALG. The percentages of MUFA and PUFA were higher in SEL than in CTRL, with greater differences in ALG and SEL/ALG ($P < 0·001$ for MUFA; $P < 0·001$ for PUFA) due to increase in trans-18:1 isomers. The percentage of MUFA was higher in SEL/ALG than in ALG ($P < 0·001$). Compared with CTRL, t10–18:1 percentage was not different in SEL but was much higher in ALG and SEL/ALG and higher in SEL/ALG than in ALG ($P < 0·001$). The percentage of t11 + c7-18:1 was higher in SEL than in CTRL, and much higher in ALG and SEL/ALG ($P < 0·001$), and did not differ between SEL/ALG and ALG. The percentage of c9–18:1 was higher in SEL than in CTRL, was lower in ALG and SEL/ALG ($P < 0·001$), and did not differ between SEL/ALG and ALG. The percentage of c9 t11 CLA was higher in SEL, was much higher in ALG and SEL/ALG ($P < 0·001$), and did not differ between SEL/ALG and ALG. The total trans-18:1 percentage was not significantly different between SEL and CTRL but was higher in ALG and much higher in SEL/ALG ($P < 0·001$). Total odd FA percentage was lower in SEL than in CTRL ($P < 0·001$), did not differ between CTRL and ALG, was higher in SEL/ALG than in CTRL ($P < 0·001$), and did not differ between SEL/ALG and ALG. The percentage of ALA was higher in SEL and SEL/ALG ($P < 0·001$) than in CTRL, was not significantly different between ALG and CTRL, and did not differ between SEL and SEL/ALG. The percentage of DHA was not significantly different between SEL and CTRL, but was higher in SEL/ALG and much higher in ALG ($P < 0·001$). The ratio of n-6/n-3 FA was lower in SEL, ALG and SEL/ALG ($P < 0·001$) than in CTRL, and was higher in SEL/ALG and SEL than in ALG ($P < 0·001$). The ratio of c9–14:1/14:0 was not significantly different between SEL and CTRL but was higher in ALG and was much higher in SEL/ALG than in CTRL ($P < 0·001$). Transfer efficiency of ALA was 2·8% for SEL and 1·4% for SEL/ALG ($P < 0·001$) and transfer efficiency of DHA was 2·7% for ALG and 2·1% for SEL/ALG ($P = 0·015$) (Table 4).

Milk fat globule size

Compared to CTRL, MFG size described by median diameter d_{50} and average diameters $d_{4,3}$ and $d_{3,2}$ was lower in ALG and SEL/ALG than in CTRL ($P < 0·001$). Average diameter $d_{3,2}$ was lower in ALG compared to SEL/ALG ($P < 0·001$) (Table 5).

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Table 3. Effects of addition of sieved EL (SEL), DHA Gold® (ALG) and sieved EL/DHA Gold®mixture (SEL/ALG) on milk yield and milk composition in dairy cows

	CTRL	SEL	ALG	SEL/ALG	RMSE	Treatment effect
Milk yield, kg/d	30·3[a]	31·5[a]	27·5[b]	24·7[c]	2·50	<0·001
Fat content, %	3·66[a]	3·45[a]	1·90[b]	1·81[b]	0·392	<0·001
Protein content, %	3·00[b]	2·87[b]	2·92[b]	3·26[a]	0·158	<0·001
Fat yield, g/d	1 118[a]	1 066[a]	515[b]	424[b]	124·0	<0·001
Protein yield, g/d	906[a]	899[a]	794[b]	793[b]	70·9	0·001
Lactose content, %	4·71	4·79	4·80	4·71	1·052	0·065
SCS[†]	2·00	2·03	2·30	2·45	0·414	0·063

RMSE, Root mean square error.
†SCS: somatic cell score = log (SCC/1 000).
[a–c]Means in the same row with no common superscript differ ($P < 0·05$).

Milk spontaneous lipolysis

Initial free FA and SL, expressed in mEq/100 g fat were higher in ALG and SEL/ALG ($P < 0·001$). Spontaneous lipolysis was higher in SEL/ALG compared to ALG ($P < 0·001$). Spontaneous lipolysis, expressed in mEq/kg of milk and in mEq/day, was higher in ALG than in CTRL (respectively, $P = 0·027$, and $P = 0·025$) (Table 5).

Discussion

Sieved extruded linseed had similar effects as standard extruded linseed

Sieved EL supplementation at 2·5% of DMI, regardless of the feed form, had no deleterious effect on milk yield, milk fat and protein content or yield. In a meta-analysis, Meignan et al. (2017) showed that standard EL supplementation increased milk yield, and decreased milk protein content regardless of diets and decreased fat content only with maize silage diets. However, in individual studies, the impacts of standard EL, at a same dose, on milk production and milk traits seem to be variable in the literature, depending on ruminal conditions (Hurtaud et al. 2010) and ruminal metabolism of lipids (Chilliard et al. 2007). Our results are consistent with those of Ferlay et al. (2013), Neveu et al. (2014) and Livingstone et al. (2015) with similar doses of standard EL in the diet.

As expected, SEL led to a decrease in SFA percentage and an increase in MUFA and PUFA percentages. The decrease in SFA was similar to that reported by Hurtaud et al. (2010) under similar condition with standard EL. The supplementation of SEL increased ALA as expected. The overall effect was an improved n-6/n-3 FA ratio in the milk. The enrichment in ALA and the transfer efficiency was consistent with Hurtaud et al. (2010) under similar conditions with standard EL (2·8% in the current study *vs.* 2·2 and 3·5%).

The supplementation of SEL had no impact on MFG size and SL. Hurtaud et al. (2010) found no impact of standard EL at 2·1% of DM on MFG size but higher level of SL. Knowing that SL is variable with milking time (Vanbergue et al. 2017), this difference is possibly due to the fact that in the current study, samples were collected during morning milkings, whereas in Hurtaud et al. (2010), samples were collected during the morning and evening milkings and pooled in a 60:40 ratio.

Sieving and sifting EL is as efficient as standard EL and can be used in ruminants' diets formulation and thus increase their sustainability, although the transfer rate from diet to milk is still low.

Microalgae DHA Gold® increased milk DHA content but induced milk fat depression

ALG and SEL/ALG led to a sharp decrease in milk yield due to a decrease in DMI and to a drastic drop in fat content and fat and protein yields. Our results are consistent with those of Boeckaert et al. (2008), AbuGhazaleh et al. (2009) and Angulo et al. (2012). These effects are similar to the effects of fish oils (Chilliard et al. 2007). Indeed, milk t10–18:1 sharply increased (+10·6%) indicating a change in ruminal fermentation and the production of fat synthesis inhibitors. The observed decrease in protein yield in ALG and SEL/ALG might be explained by the reduction in energy and protein intake.

As expected, ALG and SEL/ALG treatments led to a decrease in SFA percentage and an increase in MUFA and PUFA percentages. These changes were very significant mainly due to the significant decrease of 18:0. The decrease in SFA is consistent with results of Boeckaert et al. (2008) and Angulo et al. (2012) (except for 18:0). Fast rate of FA release into the rumen from microalgae DHA Gold® would explain higher production of trans FA, to the detriment of 18:0 production in the rumen, leading to greater inhibition of de novo mammary lipogenesis (Chilliard et al. 2009; Ferlay et al. 2013). The short FA percentage was lower with the ALG treatment than with CTRL (AbuGhazaleh et al. 2009). DHA percentage was increased by 23-fold in ALG and by 9-fold in SEL/ALG. Recovery of DHA is consistent with Boeckaert et al. (2008) (3·1% of recovery). With protected microalgae, Stamey et al. (2012) reported 3·4% of transfer efficiency and Bragaglio et al. (2015) did not detect significant change in FA profile.

ALG and SEL/ALG induced a strong decrease in MFG size. Briard-Bion et al. (2008) found that t10–18:1 was

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Table 4. Effects of sieved EL (SEL), DHA Gold® (ALG) and sieved EL/DHA Gold®mixture (SEL/ALG) on milk fatty acid composition in dairy cows. Results expressed as g/100 g total FA

	CTRL	SEL	ALG	SEL/ALG	RMSE	Treatment effect
Σ de novo FA†	29·0^{a}	26·1^{b}	25·5^{b}	22·3^{c}	1·776	<0·001
16:0	33·8^{a}	29·2^{c}	32·3^{ab}	30·3^{bc}	2·583	0·005
18:0	8·31^{b}	9·83^{a}	1·71^{c}	1·95^{c}	0·764	<0·001
t10 18:1	0·47^{c}	1·10^{c}	11·05^{b}	13·56^{a}	1·313	<0·001
t11 + c7 18:1	1·19^{c}	2·48^{b}	4·53^{a}	4·66^{a}	0·718	<0·001
c9 18:1	16·8^{b}	20·1^{a}	9·9^{c}	10·4^{c}	1·675	<0·001
c9 c12 18:2	1·57^{b}	1·50^{b}	1·85^{a}	1·62^{b}	0·214	0·041
C18:3 n-3 (ALA)	0·19^{b}	0·51^{a}	0·25^{b}	0·46^{a}	0·086	<0·001
c9 t11 CLA	0·50^{c}	1·00^{b}	1·42^{a}	1·55^{a}	0·338	<0·001
n-3 22:6 (DHA)	0·019^{c}	0·008^{c}	0·444^{a}	0·170^{b}	0·059	<0·001
ECSFA	68·5^{a}	62·7^{b}	56·5^{c}	51·4^{d}	3·25	<0·001
Short-chain FA‡	15·7^{a}	13·4^{b}	11·7^{b}	9·6^{c}	1·33	<0·001
Total odd FA	2·23^{bc}	2·03^{d}	2·35^{ab}	2·44^{a}	0·160	<0·001
BCFA	0·80^{b}	0·84^{b}	1·08^{a}	1·09^{a}	0·128	<0·001
SFA	70·7^{a}	64·7^{b}	58·8^{c}	53·9^{d}	3·24	<0·001
MUFA	23·6^{d}	29·3^{c}	32·3^{b}	36·9^{a}	2·58	<0·001
PUFA	2·72^{c}	3·60^{b}	5·13^{a}	4·67^{a}	0·600	<0·001
Total t18:1	3·1^{c}	5·4^{c}	17·9^{b}	20·4^{a}	1·30	<0·001
c9 18:1/16:0 ratio	0·51^{b}	0·71^{a}	0·32^{c}	0·34^{c}	0·099	<0·001
c9 14:1/14:0 ratio	0·108^{c}	0·109^{c}	0·149^{b}	0·209^{a}	0·024	<0·001
n-6/n-3	5·7^{a}	2·7^{cd}	2·2^{d}	2·8^{c}	0·51	<0·001

RMSE, Root mean square error; FA, fatty acid; ALA, alpha linolenic acid; DHA, docosahexaenoic acid; ECSFA, sum of even-chain saturated fatty acids; BCFA, sum of branched-chain fatty acids.
†Σ de novo FA: from C4 to C15.
‡Short-chain FA = FA < 14:0.
$^{a–d}$Means in the same row with no common superscript differ ($P < 0·05$).

negatively correlated with MFG size ($R^2 = 0·87$) and related to reduced milk fat. Smaller MFG have previously been associated with a decrease in fat content (Hurtaud et al. 2010). The decrease in milk fat content would induce synthesis of smaller MFG, as also reported by Couvreur & Hurtaud (2017). ALG and SEL/ALG also increased initial FFA and SL, expressed in mEq/100 g of fat, compared to CTRL. We showed that SL increased sharply beyond a certain threshold of microalgae DHA Gold® supplementation because SL (in mEq/kg of milk) did not differ between SEL/ALG and CTRL. According to Cartier & Chilliard (1990), MFG membrane integrity is an important factor determining SL susceptibility. Both ALG and SEL/ALG were associated with a drastic reduction in MFG size, although only ALG was associated with an increase in SL (in mEq/kg of milk). The long-chain FA profile of the MFG membrane could have differed between ALG and SEL/ALG due to differences in the FA profile in the diet. These differences could lead to differences in MFG membrane integrity.

Sieved extruded linseed and microalgae combination had a stronger impact on milk fatty acid profile and milk fat characteristics

Although the dose of DHA Gold® in SEL/ALG was half the dose used in ALG (156 *vs.* 340 g/d), the SEL/ALG treatment led to a larger decrease in milk yield and fat yield (although non-significant for fat yield) compared to ALG. The percentage of t10–18:1 was effectively higher, which would explain the more dramatic down-regulation of milk fat synthesis (Shingfield et al. 2010). The short and SFA percentages were lower and the MUFA percentages were higher with SEL/ALG than with ALG. This could be explained by the increased in trans-18:1 as previously discussed, and by the increase in the 14:1/14:0 ratio that could reflect an increase of Δ9 desaturase activity. Based on the literature available about non-protected DHA Gold® used at different doses (Boeckaert et al. 2008; AbuGhazaleh et al. 2009; Angulo et al. 2012), we assumed that the relation between the dose of DHA Gold® and milk fat depression was non-linear. Boeckaert et al. (2008) noted a fat depression of 520 and 750 g/d respectively for 382 and 195 g/d of DHA Gold® in the same conditions. The higher milk fat depression in SEL/ALG could be explained by the higher n-3 FA intake (+55·7 g/d) compared to ALG.

The $d_{3,2}$ was lower for SEL/ALG than for ALG. This could be explained by the difference in the long chain FA profile as also observed by Lu et al. (2016). Indeed, DHA and EPA were respectively 2·6 and 1·9 lower in SEL/ALG compared to ALG. Spontaneous lipolysis was also lower in SEL/ALG compared to ALG. Milk fat globule structure in relation to FA profile could explain the observed difference (Vanbergue, 2017).

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Table 5. Effects of addition of sieved EL (SEL), DHA Gold® (ALG) and sieved EL/DHA Gold® mixture (SEL/ALG) on milk fat characteristics (milk fat globule size, milk spontaneous lipolysis) in dairy cows

	CTRL	SEL	ALG	SEL/ALG	RMSE	Treatment effect
Milk fat globule						
d_{50}†, µm	3·66[a]	3·25[a]	2·21[b]	2·35[b]	0·372	<0·001
$d_{4,3}$‡, µm	3·86[a]	3·41[a]	2·35[b]	2·63[b]	0·423	<0·001
$d_{3,2}$§, µm	2·80[a]	2·36[a]	0·64[c]	0·93[b]	0·451	<0·001
Free fatty acids						
iFFA, mEq/100 g fat	0·40[b]	0·45[b]	0·95[a]	0·87[a]	0·122	<0·001
iFFA, mEq/kg milk	0·14	0·16	0·17	0·16	0·105	0·435
iFFA, mEq/day	4·21	4·66	4·66	3·94	0·133	0·762
Spontaneous lipolysis						
SL, mEq/100 g fat	0·24[c]	0·25[c]	1·12[a]	0·54[b]	0·254	<0·001
SL, mEq/kg milk	0·09[b]	0·08[b]	0·20[a]	0·10[b]	0·255	0·027
SL, mEq/day	2·53[b]	2·57[b]	5·51[a]	2·52[b]	0·268	0·025

RMSE, Root mean square error; iFFA, initial free fatty acids; SL, spontaneous lipolysis.
†d_{50} = the median diameter of milk fat globule.
‡$d_{4,3} = \Sigma(Ni \times di^4)/\Sigma(Ni \times di^3)$, volume- weighted average diameter, where Ni is the number of fat globules in a size class of diameter di.
§$d_{3,2} = \Sigma(Ni \times di^3)/\Sigma(Ni \times di^2)$, volume-surface average diameter, where Ni is the number of fat globules in a size class of diameter di.
[a–c]Means in the same row with no common superscript differ ($P < 0·05$).

Conclusion

Sieved extruded linseed had a positive impact on the milk FA profile, despite the low transfer efficiency of beneficial FA from the diet to the milk. Sieved extruded linseed supplementation at a dose of 2·5% of DMI had no deleterious effect on milk mineral and protein composition, and fat characteristics. So, it could replace standard EL to increase the sustainability of dairy cows' diets providing adapted linseed feedstuff to ruminants. Microalgae supplementation (340 and 156 g/d) had deleterious effects on milk composition, milk FA, and fat characteristics, and the effect was even more deleterious when microalgae were mixed with sieved EL. Lipid supplementation of the dairy cow diet can increase levels of valuable FA in milk if protected from rumen biohydrogenation. Protected microalgae and the doses of microalgae in the diet should be further investigated to prevent FA modification in the rumen and the consequent deleterious effects on milk fat.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S0022029918000390

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