Effectiveness of extruded rapeseed associated with an alfalfa protein concentrate in enhancing the bovine milk fatty acid composition

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ABSTRACT

Linseed and rapeseed, good sources of 18:3 n-3 and *cis*9-18:1, respectively, have been shown to improve the bovine milk fatty acid (FA) profile. However, rapeseed, unlike linseed, has little effect on the concentration of 18:3 n-3 in milk fat. Alfalfa protein concentrate (APC), besides being a valuable protein source for milk production, contains lipids rich in 18:3 n-3. Therefore, this experiment aimed at (1) evaluating the transfer efficiency of unsaturated FA (UFA), especially 18:3 n-3, of APC to bovine milk fat, and (2) evaluating whether extruded rapeseed (ER) associated with APC is as effective as extruded linseed (EL) in enhancing the bovine milk fat composition. Six lactating Holstein cows were used in a replicated 2 × 2 Latin square design with 2 iso-energy, iso-nitrogen and iso-FA corn silage-based diets (EL and ER-APC) and two 21-d periods. Extruded linseed, as main UFA source, was included in the first diet, whereas ER, as main UFA source, and APC, as supplemental 18:3 n-3, were included in the second diet. Diets were distributed as a restricted total mixed ration. Compared with the EL diet, the ER-APC diet, where ER was associated with APC, increased milk concentration of 18:3 n-3 (1.18 vs. 1.31% of FA) and *cis*9-18:1 (18.35 vs. 20.01% of FA). The apparent transfer efficiency of 18:3 n-3 from diet to milk was almost twice as much for the ER-APC diet than for the EL diet (7.4 vs. 3.8% of intake). Extruded linseed accounted for 84% of 18:3 n-3 provided in the EL diet, whereas ER and APC accounted for 33 and 38% of 18:3 n-3 provided in the ER-APC diet, respectively. Because both EL and ER underwent extrusion in similar conditions, these results suggest that 18:3 n-3 of EL in the EL diet and ER in the ER-APC diet were subjected to more extensive ruminal biohydrogenation than 18:3 n-3 of APC in the ER-APC diet. This experiment shows that corn silage-

based diets supplemented with ER as the main UFA source, associated with APC as supplemental 18:3 n-3, are as effective as corn silage-based diets supplemented with EL as the main UFA source, in increasing bovine milk UFA and 18:3 n-3 contents. Furthermore, at similar levels of dietary incorporation, this experiment shows that the ruminal bypass of 18:3 n-3 is higher for APC compared with EL.

Key words: alfalfa protein concentrate, milk fatty acid, linseed, rapeseed

INTRODUCTION

Extensive research has been done to enhance the nutritional quality of bovine milk fat through the diet, the major aim being to increase the unsaturated fatty acid (UFA), n-3 polyunsaturated fatty acid (PUFA), and conjugated linoleic acid (CLA) contents because of their bioactive properties in human health. The most common nutritional means to enhance milk UFA contents, including CLA, is to use oilseeds such as cottonseed, linseed, rapeseed, soybean, or sunflower seed in the dairy cow diet.

Among oilseeds, linseed has the highest content in 18:3 n-3, representing more than 50% of total fatty acids (FA). Feeding linseed decreases milk concentrations of short- and medium-chain FA (SMCFA) and saturated FA (SFA), and consequently, increases those of long-chain FA and UFA, more specifically 18:3 n-3 and CLA, when compared with a control diet without linseed (Gonthier et al., 2005; Akraim et al., 2007). For these reasons, linseed has become the reference in Northern and Western Europe for improving the bovine milk FA profile, and thus, has been widely used both on commercial farms and for experimental work. In Europe, rapeseed has become more available in recent years because of its increased production, whereas linseed is mainly imported. Rapeseed is a good source of *cis* (c)9-18:1, representing more than 50% of total FA. Rapeseed has been shown to improve the milk FA profile by increasing concentrations of UFA and c9-18:1

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and decreasing those of SMCFA, when compared with a control diet without rapeseed (Bayourthe et al., 2000; Collomb et al., 2004). However, rapeseed had little (Bayourthe et al., 2000) or no effect (Collomb et al., 2004) on the concentrations of 18:3 n-3 and CLA in milk fat.

Alfalfa is known to be a good source of protein and can be fed to dairy cows either as fresh, dehydrated, silage, hay, or protein concentrate. The alfalfa protein concentrate (**APC**) is obtained by heat-treatment and drying of the liquid fraction high in protein expressed from the fresh alfalfa plant. Alfalfa protein concentrate is known to be a valuable protein source for milk production because it is more resistant to ruminal degradation compared with conventional protein sources such as soybean meal (Lu et al., 1982, 1988). Interestingly, APC contains approximately 10% lipids (DM basis; INRA, 2007) rich in 18:3 n-3. Kim et al. (2009) found that a beef steer diet with APC resulted in the highest level of n-6 and n-3 PUFA in meat relative to other diets and suggested that this may relate to the rumen-protected nature of the lipids in APC.

To our knowledge, no information is available in the literature on the UFA transfer efficiency from rapeseed associated with APC to milk fat in lactating dairy cows. Therefore, the objective of the present experiment was (1) to evaluate the transfer efficiency of UFA, especially 18:3 n-3, of APC to bovine milk fat, and (2) to evaluate whether extruded rapeseed (**ER**) associated with APC is as effective as extruded linseed (**EL**) in improving the bovine milk fat composition.

MATERIALS AND METHODS

Experimental Design, Animals, and Management

Six lactating Holstein cows were blocked according to their milk yield and assigned to 2 dietary treatments in a replicated 2 × 2 Latin square design. Each experimental period lasted 21 d. At the beginning of the experiment, cows averaged 3 ± 0.8 lactations, 162 ± 44.4 DIM, 636 ± 25.3 kg of BW, and yielded 30.1 ± 4.32 kg of milk/d (mean ± SD). Cows were housed in individual stalls. The floor of the stalls was bedded with mats and sawdust and cleaned twice daily. Cows were fed individually twice daily at 0830 and 1730 h and had free access to water and mineral blocks. Milking took place twice daily at 0730 and 1630 h. The experiment was carried out at the Centre wallon de Recherches agronomiques (Gembloux, Belgium) from April 23 to June 3, 2009, and was in accordance with the recommendations on care and use of laboratory animals of the Université catholique de Louvain.

Experimental Diets

Two diets (EL and ER-APC) were formulated to provide similar forage-to-concentrate ratios (60:40) and similar amounts of energy, nitrogen, and FA. The ingredient composition of the experimental diets is given in Table 1. Diets contained corn silage and grass hay as forages in the same proportions, soybean meal (in the EL diet) or APC (in the ER-APC diet) and urea as sources of nitrogen, sugar beet pulp as a source of energy, EL (in the EL diet) or ER and APC (in the ER-APC diet) as sources of supplemental UFA, a mineral and vitamin mix, ground chalk, and a vitamin E preparation to avoid milk oxidation (Focant et al., 1998). The DM content and chemical composition of the main individual feedstuffs are given in Table 2. Extruded linseed, supplied as Nutex (Dumoulin, Seilles, Belgium) consisted of an extruded mixture of linseed, wheat, sunflower cake, field beans, peas, butylated hydroxytoluene (BHT) and salt (58.5, 15.0, 14.0, 5.0, 5.0, 2.0 and 0.4% of total raw materials, respectively). The extrusion of the linseed mixture was performed with a one-screw Wenger extruder (Wenger Manufacturing Inc., Sabetha, KS) at 108°C after pre-conditioning with steam at 96°C for 3 min to adjust the moisture content of the raw linseed mixture to 22%. The total FA of EL was 23.6% of DM, with the main FA being 18:3 n-3, c9-18:1 and 18:2 n-6 (52, 21, and 17% of identified FA, respectively). Extruded rapeseed, supplied as Colmex (Dumoulin), consisted of an extruded mixture of rapeseed, wheat, BHT and salt (70.0, 28.0, 1.5, and 0.5% of total raw material, respectively). The extrusion of the rapeseed mixture was performed in the same conditions as described above for EL. The total FA of ER was 30.0% of DM, with the main FA being c9-18:1, 18:2 n-6, and 18:3 n-3 (55, 22, and 12% of identified FA, respectively). Alfalfa protein concentrate, supplied as Extraluz (Désialis, Paris, France), was obtained as follows. A liquid fraction (green juice) rich in protein was cold-expressed from fresh alfalfa. It was then treated for the flocculation of all the pigmented and cytoplasmic proteins contained in it by (1) alkalization to pH 8.5 through injection of NH₃, followed by (2) thermal flocculation by raising the temperature of the juice to 85 to 90°C (2 min) through the use of a steam injector. The protein-lipid coagulum was then centrifuged to separate the green cream (>40% of DM) from the liquid effluent (serum). The green cream was then pelletized and finally dried by hot air fluidization to obtain APC in the form of green dehydrated pellets. The CP of the APC was 58.2% of DM. The total FA of the APC was 6.7% of DM, with the main FA being 18:3 n-3, 16:0 and 18:2 n-6 (47, 18, and 17% of identified FA, respectively).

Table 1. Ingredient composition (% DM) of the 2 experimental diets¹

Ingredient	Diet ²	
	EL	ER-APC
Corn silage	50.0	50.9
Grass hay	10.2	10.2
Sugar beet pulp	15.7	20.8
Soybean meal	13.0	0.0
Alfalfa protein concentrate ³	0.0	9.3
Extruded linseed ⁴	9.3	0.0
Extruded rapeseed ⁵	0.0	6.9
Urea ⁶	0.5	0.9
Mineral and vitamin mix ⁷	0.9	0.9
Ground chalk ⁸	0.5	0.0

¹The two experimental diets received a vitamin E preparation distributed in the form of a powder containing 50% of α -tocopheryl acetate (Dumoulin, Seilles, Belgium) added to the TMR; the amount was calculated to deliver 10,000 IU/d of unprotected vitamin E to the diet.

²EL = extruded linseed diet; ER-APC diet = extruded rapeseed-alfalfa protein concentrate diet.

³Commercial alfalfa protein concentrate (Extraluz; Désialis, Paris, France).

⁴Extruded commercial concentrate (Nutex; Dumoulin) made of linseed, wheat, sunflower cake, field beans, peas, butylated hydroxytoluene (BHT), and salt (58.5, 15.0, 14.0, 5.0, 5.0, 2.0, and 0.4% of total raw materials, respectively).

⁵Extruded commercial concentrate (Colmex; Dumoulin) made of rapeseed, wheat, BHT, and salt (70.0, 28.0, 1.5, and 0.5% of total raw material, respectively).

⁶Urea: 98.0% of DM and 287.5% of CP on a DM basis (INRA, 2007).

⁷Declared contents: 15% Ca, 5% Mg, 5% P, 1% Na, 7,750 mg of Zn/kg, 3,300 mg of Mn/kg, 1,250 mg of Cu/kg, 100 mg of I/kg, 50 mg of Co/kg, 20 mg of Se/kg, 1,200,000 IU of vitamin A/kg, 200,000 IU of vitamin D₃/kg, 15,000 mg of choline/kg, 2,000 mg of vitamin E/kg, 500 mg of vitamin B₁/kg, 255 mg of vitamin B₃/kg, 75 mg of vitamin B₂/kg, 15 mg of vitamin B₆/kg, 15 mg/kg of vitamin K (Celemin; Dumoulin).

⁸Ground chalk: 99.5% of DM and 99% of crude ash on a DM basis (Dumoulin).

Diets were formulated to meet energy and protein requirements of cows according to the French Unité Fourragère Lait and Protéines Digestibles dans l'Intestin systems using the INRAtion software (version 3.21;

INRA, Paris, France) for a cow of 650 kg yielding 30 kg of milk/d. The amounts of diets were distributed as a restricted TMR to control intakes and were adapted to the individual milk production of cows.

Measurements

Individual feedstuffs were sampled at the end of each period and analyzed for DM and chemical composition. Individual cow refusals were collected during the last week of each period and analyzed for DM content. Individual cow intakes during the last week of each period were calculated for each ingredient as the amounts offered minus refusals, on a DM basis. Means of individual cow intakes calculated during the last week of each period were included in the statistical analysis. Individual cow milk yield was recorded daily during each milking. Means of individual cow milk yields recorded during the last week of each period were included in the statistical analysis. Milk samples from individual cows were collected on d 19 and 21 of each period at the a.m. and p.m. milkings. The a.m. and p.m. milk samples of each of the 2 d from individual cows were mixed in a proportional basis for analysis. These samples were used for the statistical analysis. Cows were weighed at the start and at the end of the experiment.

Chemical Analyses of Feedstuffs

Fresh corn silage and grass hay samples were analyzed for DM content by oven drying at 105°C for 48 h. The chemical composition of corn silage was determined using lyophilized samples. All feed samples were ground in a mill [1-mm mesh; Retsch, Düsseldorf, Germany; adapted from AOAC method 950.02 (AOAC, 1995)] and stored at 4°C in hermetic boxes until analysis. They were then analyzed for DM content by oven

Table 2. Dry matter content and chemical composition of the main individual feedstuffs

Item	Corn silage	Grass hay	Sugar beet pulp	Soybean meal	Alfalfa protein concentrate	Extruded linseed	Extruded rapeseed	Mineral and vitamin mix
DM, %	34.7	87.5	89.0	89.0	92.7	96.1	91.6	94.7
Chemical composition, % of DM								
Crude ash	4.2	6.8	5.7	7.2	8.5	4.7	3.9	61.6
CP	8.4	8.6	8.3	51.0	58.2	20.9	17.9	6.0
NDF	43.2	72.0	40.9	18.9	16.1	34.6	29.5	29.6
ADF	23.7	39.1	24.9	11.5	2.7	17.8	14.3	5.5
Crude fat	7.3	3.5	3.5	4.3	17.6	26.3	34.9	4.1
Fatty acid								
Total fatty acids	2.3	0.8	1.7	1.8	6.7	23.6	30.0	1.2
6:0–14:0	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.1
16:0	0.4	0.2	0.3	0.3	1.2	1.4	1.6	0.3
18:0	0.1	0.0	0.0	0.1	0.2	0.9	0.5	0.1
cis-9-18:1	0.5	0.1	0.2	0.3	0.3	5.0	16.4	0.4
18:2 n-6	0.9	0.1	0.6	1.0	1.1	4.0	6.5	0.2
18:3 n-3	0.3	0.2	0.5	0.1	3.1	12.2	3.6	0.0

drying at 105°C for 16 h [adapted from AOAC methods 967.03 and 930.15 (AOAC, 1995)], crude ash by ashing at 550°C for 16 h [adapted from AOAC methods 923.03, 967.04, and 942.05 (AOAC, 1995)], CP by the Kjeldahl method [$N \times 6.25$; adapted from AOAC methods 981.10 and 991.20 (AOAC, 1995)], crude fat (adapted from directive 98/64/CE; Commission des Communautés européennes, 1998), NDF, and ADF (Van Soest et al., 1991). Neutral detergent fiber and ADF were determined sequentially and were expressed without residual ash. Neutral detergent fiber was analyzed with the addition of α -amylase and without sodium sulfite. For the determination of FA profiles, lipids were extracted following the method of Folch modified by Christie (1982) and 13:0 (Sigma-Aldrich, St. Louis, MO) was used as an internal standard. Methylation and GLC analyses were performed as described below for milk samples.

In Vitro Determination of Biohydrogenation Percentages of UFA in Rumen Fluid

Ninety-five milligrams of EL, 75 mg of ER, or 330 mg of APC was added into 100-mL flasks containing 400 mg of grass hay as a source of feed particles. The amounts of EL, ER, and APC were calculated based on their total FA content (Table 2) to provide the same amount of FA for each dietary treatment. Ruminant fluid was obtained from 3 ruminally cannulated dry Holstein cows receiving grass hay ad libitum plus 1.5 kg/d of a concentrate (2/3 barley + 1/3 soybean meal) for 3 wk. Cows were housed in individual stalls, fed twice daily, and provided with water ad libitum. Ruminant fluid from each of the cows was collected before the a.m. meal, mixed together in equal proportions, strained through a metal sieve (0.4 mm mesh), and finally brought to the laboratory under anaerobic conditions at 39°C. The ruminal fluid mixture from the 3 cows was then mixed in a ratio 1:2 (vol:vol) with a buffer solution containing (g/L) NaHCO_3 , 6.533; $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 6.200; KCl, 0.380; NaCl, 0.313; CaCl_2 , 0.080; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.080, pre-warmed at 39°C and saturated with CO_2 (pH 6.9). The mixture was then gassed with CO_2 to maintain anaerobic conditions. Fifty milliliters of the mixture was transferred to each flask of the experimental set. Flasks were gassed with CO_2 before closing, placed in a shaking water bath (160 rpm, 39°C) and incubated for 0, 3, 6, 18, or 24 h. Flasks were incubated in triplicate for each treatment and each incubation time. Incubations were stopped by placing the flasks into iced water. Five milliliters of the homogenized content was collected and lyophilized. Fatty acids in the lyophilized samples were extracted and methylated in one step (Park and Goins, 1994) for FA analysis by GLC. Conditions for GLC analysis were as described below for milk samples.

Milk Analyses

Milk samples were refrigerated at 4°C. A portion of each sample was used for fat, CP, and urea analyses by the mid-infrared spectroscopic method (adapted from AOAC method 972.16; AOAC, 1995) using the MilkoScan FT6000 spectrophotometer (Foss Analytical, Hillerød, Denmark) at the certified Milk Control Station [Battice, Belgium; Belgian accreditation number 262-TEST in compliance with International Organization for Standardization (ISO) 17025], and the rest of each sample was used for fat extraction.

Lipids from milk samples were extracted following the method described by Hara and Radin (1978). Milk samples were centrifuged at $17\,800 \times g$ at 4°C for 30 min, and 300 to 350 mg of the fat cake was collected. Lipid extraction from milk fat was performed with 7.2 mL of a hexane/isopropanol (3:2, vol:vol) solution, followed by 4.8 mL of an Na_2SO_4 solution (67 g/L). The upper phase was removed and dried with 1 g of anhydrous Na_2SO_4 . It was then transferred again and brought to dryness under a continuous stream of N_2 . Fatty acids were first hydrolyzed in a solution of KOH in methanol (0.1 M) at 70°C for 60 min, then methylated in a solution of HCl in methanol (1.2 M) at 70°C for 20 min, and finally extracted with hexane.

Fatty acid methyl esters were separated and quantified with GLC (ThermoQuest Trace GC; Thermo Finnigan, Milan, Italy) equipped with a flame ionization detector, an automatic injector, and a fused silica capillary column (100 m $\times$ 0.25 mm i.d.), coated with a 0.2- μm film of biscyanopropyl polysiloxane (Rt-2560; Restek Corp., Bellefonte, PA). The system used H_2 as the carrier gas operated at a constant pressure of 200 kPa. Injection was on column so as to inject the entire sample into the column head. This injection mode was preferred to the split-injection mode because it minimizes the risk of discrimination between milk FA with very different volatility. The initial oven temperature was 80°C; it increased at 25°C/min to 175°C (held for 25 min), then increased at 10°C/min to 205°C (held for 4 min), then increased at 10°C/min to 225°C (held for 20 min), and finally decreased at 20°C/min to 80°C. The temperature of the flame ionization detector was maintained at 255°C. Hydrogen flow to the detector was 35 mL/min and air flow was 350 mL/min. Each peak, except that of *trans* (*t*)10-18:1, was identified and quantified by comparison of retention times with pure fatty acid methyl ester (FAME) standards (Alltech Associates Inc., Deerfield, IL; except CLA isomers from Nu-Chek Prep Inc., Elysian, MN). Because no commercial standard was available for *t*10-18:1, the concentration of the corresponding peak was calculated by comparison with the *t*11-18:1 peak area (by multiplying the con-

concentration of *t*11-18:1 by the *t*10 peak area-to-*t*11 peak area ratio). The validity of this method was verified by applying it to the peak of *t*9-18:1 and comparing this calculated concentration with the *t*9-18:1 concentration determined through the use of the appropriate standard (Alltech Associates Inc.). Each FA was expressed as a percentage of all identified FA. The total identified FA accounted for $94.1 \pm 1.13\%$ of the total measured FA (mean $\pm$ SD), as calculated by the ratio between the total area of identified peaks and the total area of identified and unidentified peaks. The analysis method did not allow quantification of 4:0, which is present in milk fat at $\pm 3\%$ of the total theoretical FA (Jensen, 2002). Therefore, the total identified FA would account for $\pm 91\%$ of the total theoretical FA. According to the literature, the *c*9,*t*11 CLA peak contained *c*9,*t*11 CLA and minor proportions of *t*7,*c*9 and *t*8,*c*10 CLA (Cruz-Hernandez et al., 2006).

Calculations

The in vitro biohydrogenation percentage (%) of a given FA at incubation time *t* was calculated as

$$100 - \frac{\text{Concentration of FA at time } t \times 100}{\text{Concentration of FA at time } 0}.$$

Individual milk FA yield (g/d) was calculated as

$$\begin{aligned} \text{Individual milk FA yield} &= \text{milk fat yield} \\ &\times \text{individual milk FA}\% / 100 \times 0.933 \times 0.91, \end{aligned}$$

where 0.933 is the mean proportion of FA in milk fat estimated by Glasser et al. (2007), 0.91 is the proportion of the identified FA in the total theoretical FA, and milk fat yield is expressed in g/d.

The apparent transfer efficiency of 18:2 n-6 and 18:3 n-3 from diet to milk could be estimated by dividing the milk 18:2 n-6 or 18:3 n-3 yield (g/d) by its dietary intake (g/d).

Statistical Analysis

All data from the in vitro determination of biohydrogenation percentages of UFA in rumen fluid were analyzed using the ANOVA procedure of SAS (version 9.1.3; SAS Institute Inc., Cary, NC). Overall, differences between treatment means at each time of incubation were considered to be significant when $P < 0.05$. When a significant treatment effect was observed, the Tukey option was used to compare means.

Data on milk composition and milk FA composition were averaged over d 19 and 21 of each period before

statistical analyses. All data from the experiment are reported as least squares means $\pm$ standard error of the means and were analyzed as a replicated Latin square using the MIXED procedure of SAS (version 9.1.3; SAS Institute Inc.). The statistical model included period, cow, diet, and random error. Fixed effects included period and diet. Cow was the random effect. Overall differences between treatment means were considered to be significant when $P < 0.05$.

RESULTS

In Vitro UFA Ruminal Biohydrogenation Percentages of Extruded Linseed, Extruded Rapeseed, and Alfalfa Protein Concentrate

In vitro ruminal biohydrogenation percentages of 18:3 n-3, 18:2 n-6, and *c*9-18:1 at different incubation times are shown in Figure 1 for EL, ER, and APC. Biohydrogenation percentages of 18:3 n-3, 18:2 n-6, and *c*9-18:1 were similar for EL and ER at all time points, and higher than those of APC after 18 h of incubation ($P < 0.05$). At the end of the incubation (24 h), biohydrogenation percentages of 18:3 n-3 were 92, 91, and 67%; those of 18:2 n-6 were 86, 94, and 52%; and those of *c*9-18:1 were 68, 84, and 33% for EL, ER, and APC, respectively.

Nutrient Intake

Nutrient intake of cows fed the 2 experimental diets is summarized in Table 3. Individual cow refusals throughout the experiment averaged $4 \pm 3.9\%$ (mean $\pm$ SD) of offered DM. Because all individual cow refusals collected for each period were grass hay, refusals were only analyzed for DM content and the amount of grass hay refusals was subtracted from the amount of grass hay offered (DM basis). The amounts of diets were distributed as restricted TMR to control intakes. Accordingly, intakes of DM, OM, and NDF did not differ among diets ($P > 0.05$) and averaged 23.2, 21.8, and 9.7 kg/d, respectively. The intake of CP was higher for the EL diet compared with the ER-APC diet ($P = 0.03$). On the contrary, the intake of crude fat was higher for the ER-APC diet compared with the EL diet ($P = 0.01$). However, in agreement with the design of this experiment, the total FA intake did not differ among diets ($P > 0.05$) and averaged 925 g/d. The discrepancy between crude fat and total FA intakes for the 2 diets was due to the low proportion of total FA in crude fat for APC (0.38) compared with EL and ER (0.90 and 0.86; Table 2). Differences appeared between the 2 diets in terms of intakes of some FA, mainly *c*9-18:1 and 18:3 n-3. As a result, 18:3 n-3 was the main

Figure 1. In vitro ruminal biohydrogenation percentages of 18:3 n-3 (a), 18:2 n-6 (b), and *cis*-9-18:1 (c) for extruded linseed (solid line), extruded rapeseed (dashed line), and alfalfa protein concentrate (dotted line). Error bars indicate standard error of the means (n = 3). Means marked with * differ significantly ($P < 0.05$).

FA consumed in the EL diet, followed by 18:2 n-6 and *c*9-18:1 (35, 27, and 20% of total FA intake, respectively), whereas *c*9-18:1 was the main FA consumed in the ER-APC diet, followed by 18:2 n-6 and 18:3 n-3 (33, 28, and 19% of total FA intake, respectively). Cows did not lose weight during the experiment (636 ± 25.3 vs. 642 ± 31.2 kg of BW, respectively at the onset and at the end of the experiment), suggesting that energy requirements were met.

Milk Yield and Composition

Milk yield and composition of cows fed the 2 experimental diets are shown in Table 4. Milk, 4% FCM, and fat yields were not affected by diets ($P > 0.05$) and averaged 28.7, 29.3, and 1.19 kg/d, respectively. The efficiency of milk production, in terms of 4% FCM yield (kg) per kilogram of DMI, did not differ among diets ($P > 0.05$) and averaged 1.27. Fat and protein percentages were not affected by diets ($P > 0.05$) and averaged 4.21 and 3.21%, respectively. On the contrary, the protein yield was higher for the EL diet compared with the ER-APC diet ($P = 0.02$). However, this difference could be considered as having no or negligible biological significance in spite of having statistical significance. The milk urea concentration was lower for the ER-APC diet compared with the EL diet ($P = 0.01$).

Milk FA Composition and Yield

The milk FA composition of cows fed the 2 experimental diets is shown in Table 5. Milk concentrations of 16:0, 18:0, *t*10-18:1, PUFA, UFA, SFA, and C20-C22 did not differ between the EL and ER-APC diets ($P > 0.05$). Milk concentrations of 6:0 to 14:0; *t*11-18:1; *c*9,*t*11 CLA, and total *trans* C18 FA were higher for the EL diet compared with the ER-APC diet. On the contrary, milk concentrations of *c*9-18:1, 18:2 n-6, 18:3 n-3, total C18 FA, and monounsaturated fatty acids (MUFA) were higher for the ER-APC diet compared with the EL diet.

The milk FA yields of cows fed the 2 experimental diets are shown in Table 6. Milk FA yields did not differ among diets ($P > 0.05$), except for *c*9,*t*11 CLA and total *trans* C18 FA, whose yields were higher for the EL diet. The transfer efficiency of 18:3 n-3 in milk was almost twice as much for the ER-APC diet as for the EL diet. The transfer efficiency of 18:2 n-6 in milk did not differ among diets ($P > 0.05$).

DISCUSSION

Compared with the FA composition of a winter Dutch milk (mean from 1,822 milk samples from individual cows between February and March 2005; Rutten et al., 2009) and a year-round Dutch milk (mean from 52 bulk milk samples from 17 dairy plants collected every week between February 2005 and February 2006; Heck et al., 2009), milks from the EL diet (EL milk) and the ER-APC diet (ER-APC milk) exhibited higher concentrations of 18:3 n-3 (1.18 and 1.31 vs. 0.42 and 0.50% of FA, respectively, for EL, ER-APC, winter, and year-round milks); *c*9,*t*11 CLA (1.24 and 1.00 vs. 0.39 and 0.54% of FA, respectively); and lower ratios

Table 3. Nutrient intake of cows fed the 2 experimental diets

	Diet ¹			
Intake	EL	ER-APC	SEM	<i>P</i> -value
Nutrient intake, kg/d				
DM	23.6	22.7	0.37	NS
OM	22.2	21.3	0.35	NS
Crude ash	1.4	1.4	0.02	NS
CP	3.7	3.5	0.06	0.03
NDF	9.8	9.5	0.15	NS
ADF	5.5	5.1	0.08	0.02
Crude fat	1.8	2.0	0.03	0.01
Fatty acid intake, g/d				
Total fatty acids	918	931	15.6	NS
Total C18 fatty acids	785	761	12.9	NS
6:0–14:0	10	11	0.2	<0.01
16:0	102	112	1.8	0.01
18:0	30	20	0.4	<0.01
<i>cis</i> -9-18:1	185	311	4.9	<0.01
18:2 n-6	252	258	4.2	NS
18:3 n-3	318	172	3.7	<0.01

¹EL = extruded linseed diet; ER-APC = extruded rapeseed-alfalfa protein concentrate diet.

of 18:2 n-6/18:3 n-3 (1.69 and 1.64 vs. 2.83 and 2.56, respectively). Moreover, when compared with the same winter (Rutten et al., 2009) and year-round (Heck et al., 2009) milks, the EL and ER-APC milks exhibited higher concentrations of t11-18:1 (2.99 and 2.37 vs. 0.78 and 1.12% of FA, respectively), whose presence in milk fat may have a positive effect on some aspects of human health because human tissues can partially convert t11-18:1 to c9,t11 CLA via Δ^9 -desaturase (Renaville et al., 2006). To a lesser extent, when compared with winter milk (Rutten et al., 2009), the EL and ER-APC milks exhibited lower concentrations of 16:0 (29.35 and 28.77 vs. 32.61% of FA, respectively, for EL, ER-APC, and winter milks) and 12:0 (3.57 and 3.70 vs. 4.10% of FA, respectively), and lower ratios of SFA/UFA (2.32 and 2.21 vs. 2.81, respectively). Besides, the ER-APC milk exhibited a higher concentration of c9-18:1 compared with the winter (Rutten et al., 2009) and year-round (Heck et al., 2009) milks (20.01 vs. 18.03 and 17.60% of FA, respectively, for ER-APC, winter and year-round

milks). Differences in terms of milk FA concentrations between winter and year-round milks, on the one hand, and EL and ER-APC milks, on the other hand, can be partly attributed to differences in FA intakes. The major ingredients in Dutch dairy cow rations include fresh grass, grass silage, corn silage, and concentrates, and the lipids present in these feedstuffs usually account for only 2 to 4% of the diet (DM basis), whereas in EL and ER-APC diets, the lipid inclusion, and subsequent FA intake, was much higher (7.6 to 8.8 and 3.9 to 4.1% of DM, respectively; calculated from Table 3), due to the supplementation with EL, ER, and APC. Regarding UFA, c9-18:1 and SMCFA, these observations are in line with those of Gonthier et al. (2005) and Akraim et al. (2007) for linseed supplementation, and Bayourthe et al. (2000) and Collomb et al. (2004) for rapeseed supplementation. However, rapeseed only had little (Bayourthe et al., 2000) or no effect (Collomb et al., 2004) on the concentration of 18:3 n-3 in milk fat. The level of lipid inclusion in the diet might, however, not

Table 4. Milk yield and composition of cows fed the 2 experimental diets

Item	Diet ¹		SEM	<i>P</i> -value
	EL	ER-APC		
Milk, kg/d	29.4	28.0	1.47	NS
4% FCM, kg/d	30.0	28.6	0.99	NS
Fat, kg/d	1.22	1.16	0.043	NS
Protein, kg/d	0.94	0.88	0.037	0.02
Fat, %	4.24	4.18	0.242	NS
Protein, %	3.22	3.20	0.058	NS
Urea, mg/L	233	166	11.3	0.01
Efficiency of milk production, kg of 4% FCM/kg of DMI	1.27	1.26	0.039	NS

¹EL diet = extruded linseed diet; ER-APC diet = extruded rapeseed-alfalfa protein concentrate diet.

Table 5. Milk fatty acid composition (% identified fatty acids) of cows fed the 2 experimental diets

Fatty acid ¹	Diet ²		SEM	P-value
	EL	ER-APC		
6:0	2.70	2.59	0.093	NS
8:0	1.63	1.54	0.066	0.01
10:0	3.38	3.16	0.149	0.01
12:0	3.57	3.70	0.144	0.02
13:0	0.17	0.17	0.016	NS
14:0	12.35	11.98	0.411	0.03
Iso-14:0	0.09	0.08	0.007	NS
c9-14:1	1.02	1.00	0.085	NS
15:0	0.99	1.09	0.029	NS
Iso-15:0	0.25	0.20	0.005	0.01
Anteiso-15:0	0.58	0.57	0.030	NS
16:0	29.35	28.77	1.644	NS
Iso-16:0	0.29	0.34	0.027	NS
c9-16:1	1.30	1.34	0.205	NS
t9-16:1	0.16	0.14	0.021	NS
17:0	0.57	0.61	0.015	0.03
Iso-17:0	0.34	0.27	0.021	0.01
Anteiso-17:0	0.59	0.55	0.040	0.04
18:0	12.46	12.96	0.767	NS
c9-18:1	18.35	20.01	0.542	0.01
c11-18:1	0.47	0.73	0.037	0.01
t9-18:1	0.40	0.54	0.038	0.01
t10-18:1	0.54	0.72	0.071	NS
t11-18:1	2.99	2.37	0.408	0.03
18:2 n-6	1.96	2.11	0.193	0.03
c9,t11 CLA ³	1.24	1.00	0.160	0.01
t9,t12-18:2	0.33	0.12	0.037	0.01
18:3 n-3	1.18	1.31	0.154	0.04
20:0	0.12	0.20	0.012	0.01
c11,c14,c17-20:3	ND ⁴	ND	NA ⁵	NA
c5,c8,c11,c14-20:4	0.07	0.06	0.014	NS
c5,c8,c11,c14,c17-20:5	0.45	0.05	0.304	NS
c7,c10,c13,c16,c19-22:5	0.08	0.08	0.021	NS
c4,c7,c10,c13,c16,c19-22:6	ND	ND	NA	NA
6:0-14:0	23.79	22.81	0.787	0.01
Total <i>trans</i> C18 fatty acids	5.53	4.75	0.643	0.03
Total C18 fatty acids	39.95	41.87	1.552	0.01
C20-C22	0.60	0.18	0.301	NS
Monounsaturated fatty acids	25.24	26.84	1.006	0.01
Polyunsaturated fatty acids	5.33	4.72	0.722	NS
Saturated fatty acids	69.44	68.44	1.644	NS
Unsaturated fatty acids	30.56	31.56	1.644	NS
18:2 n-6/18:3 n-3	1.69	1.64	0.051	NS
Saturated fatty acids/unsaturated fatty acids	2.32	2.21	0.168	NS

¹c = *cis*; t = *trans*.²EL = extruded linseed diet; ER-APC = extruded rapeseed-alfalfa protein concentrate diet.³The GLC analysis was unable to separate the minor quantities of t7,c9 and t8,c10 conjugated linoleic acid (CLA) isomers from the main c9,t11 CLA isomer.⁴Not detected.⁵Not applicable.

totally explain the differences in milk UFA observed between regular Dutch milks and the ones obtained in the present experiment. A partial protection from thorough biohydrogenation also appears to play a role. The *in vitro* biohydrogenation percentages presented in Figure 1 indicate that the UFA from APC were less completely biohydrogenated than those from EL and ER. In line with these *in vitro* data, the results obtained with the ER-APC diet, where rapeseed was associated

with APC, showed a higher milk concentration of 18:3 n-3, in addition to the increase in UFA and c9-18:1 and decrease in SMCFA induced by rapeseed. Overall, our results show that EL and ER +APC, incorporated as UFA sources into corn silage-based diets, are 2 effective means of improving the milk FA profile.

In this experiment, milk concentrations of c9,t11 CLA and t11-18:1, the main biohydrogenation intermediates, were higher for the EL diet compared with

Table 6. Milk fatty acid yields of cows fed the 2 experimental diets

Item ¹	Diet ²		SEM	P-value
	EL	ER-APC		
Fatty acid yield, g/d				
6:0–16:0	551	508	30.3	NS
18:0	128	128	9.0	NS
<i>c</i> 9–18:1	190	196	6.9	NS
<i>t</i> 11–18:1	31	23	3.5	NS
18:2 n-6	20	21	1.7	NS
<i>c</i> 9, <i>t</i> 11 CLA ³	13	10	1.5	0.01
18:3 n-3	12	13	1.3	NS
Total <i>trans</i> C18 fatty acids	57	46	5.6	0.04
Total C18 fatty acids	412	411	15.6	NS
Saturated fatty acids	719	675	35.3	NS
Unsaturated fatty acids	315	309	14.7	NS
Total fatty acids	1,034	983	36.9	NS
Transfer efficiency from diet to milk, % intake				
18:2 n-6	8.0	8.0	0.63	NS
18:3 n-3	3.8	7.4	0.62	<0.01

¹*c* = *cis*; *t* = *trans*.²EL diet = extruded linseed diet; ER-APC diet = extruded rapeseed-alfalfa protein concentrate diet.³The GLC analysis was unable to separate the minor quantities of *t*7,*c*9 and *t*8,*c*10 conjugated linoleic acid (CLA) isomers from the main *c*9,*t*11 CLA isomer.

the ER-APC diet. This might partly be because of the higher protection of UFA provided by APC in the ER-APC diet. Quantitatively speaking, the main reason for that difference is most likely the higher intake of PUFA with the EL diet (despite similar total FA intake). Accordingly, Rego et al. (2009) showed that higher milk concentrations of biohydrogenation intermediates were obtained with linseed oil (source of 18:3 n-3) compared with rapeseed oil (source of *c*9–18:1), and the lack of effect on *t*11–18:1, and subsequently on *c*9,*t*11 CLA in milk fat, of rapeseed supplementation suggested that *t*11–18:1 is not the main product of rumen microbial isomerization of *c*9–18:1.

In the present study, extruded material was used either from linseed or from rapeseed. The extrusion process has been claimed to offer a certain level of protection to the UFA. It has been proposed that this process would denature the protein matrix that might have encapsulated or tied up FA, and in turn, make them less available for ruminal biohydrogenation (Kennelly, 1996; Petit et al., 1997). Moreover, the temperature of preconditioning and the increase in temperature between the preconditioning and extrusion steps have also been reported to strongly influence oil availability and subsequent milk FA profile (Chesneau et al., 2009). As an example, Akraim et al. (2007) showed that extruded, compared with raw, ground linseed leads to increased milk proportions of 18:3 n-3 and ruminal biohydrogenation intermediates, including *c*9,*t*11 CLA. However, reported effects of extrusion processes on ruminal degradability of oilseeds are inconsistent and hardly complete. Indeed, some authors showed that

extruded oilseeds decreased UFA ruminal biohydrogenation compared with raw oilseeds (Chapoutot and Sauvant, 1997; Chouinard et al., 1997; Akraim et al., 2007), whereas others did not come to the same conclusions (Gonthier et al., 2005; Akraim et al., 2006; Doreau et al., 2009).

The transfer efficiency of 18:3 n-3 into milk was almost twice as much for the ER-APC diet as for the EL diet. Based on the proportion of EL in the EL diet (Table 1), the proportion of 18:3 n-3 in EL (Table 2) and the calculated proportion of 18:3 n-3 in the EL diet as consumed (Table 3), we estimated that EL accounted for approximately 84% of the 18:3 n-3 provided in the EL diet. With the same approach, ER and APC accounted for approximately 33 and 38% of 18:3 n-3 provided in the ER-APC diet, respectively. Both EL and ER underwent extrusion in similar conditions, resulting in their *in vitro* UFA ruminal biohydrogenation percentages being similar. *In vitro* data also indicated that the UFA from APC, and thus 18:3 n-3, were partially protected from biohydrogenation. Taken together, our results, therefore, suggest that 18:3 n-3 of EL in the EL diet and ER in the ER-APC diet were subjected to more extensive ruminal biohydrogenation than 18:3 n-3 of APC in the ER-APC diet. Another explanation might be the difference in 18:3 n-3 levels in the 2 experimental diets. Akraim et al. (2007) suggested that the extent of ruminal biohydrogenation of 18:3 n-3 is increased when its dietary supply was high, which was the case for the EL diet. The transfer efficiency of 18:2 n-6 into milk did not differ among diets. This was expected because the main source of 18:2 n-6 for the 2 diets was corn

silage, which accounted for approximately 42 and 40% provided in the EL and ER-APC diets, respectively. Extruded linseed accounted for approximately 34% of 18:2 n-6 provided in the EL diet, whereas ER and APC accounted for approximately 39 and 9% of 18:2 n-6 provided in the ER-APC diet, respectively. Because APC was a minor source of 18:2 n-6, a potential influence of partially protected 18:2 n-6 from APC on its milk level would not have been noticed. In contrast, the major sources of 18:2 n-6, which were EL in the EL diet and ER in the ER-APC diet, were most likely equally subjected to ruminal biohydrogenation. The transfer efficiency of c9-18:1 in milk could not be assessed because mammary Δ^9 -desaturation of 18:0 is responsible for 80% of the yield of c9-18:1 in milk (Glasser et al., 2008).

Heat coagulation and dehydration during the alfalfa plant fractionation process increase ruminal bypass of alfalfa proteins (Lu et al., 1982; Nishino et al., 1994) and may, subsequently, protect alfalfa lipids from ruminal biohydrogenation by a mechanism similar to that of extruded oilseeds. Furthermore, differences of 18:3 n-3 transfer efficiencies from diet to milk observed for EL and ER-APC diets, together with in vitro biohydrogenation percentages of 18:3 n-3, 18:2 n-6, and c9-18:1 for EL, ER, and APC, suggest that the level of protection of UFA from ruminal biohydrogenation provided by the heat treatment (temperature and duration) as well as the source of protein of EL and ER was lower than that of APC.

In this experiment, soybean meal, as the main protein source in the EL diet was totally replaced by APC in the ER-APC diet. The digestible proteins from dietary origin entering the small intestine (PDIA; directly originating from proteins that are not degraded in the rumen; INRA, 2007) values were higher for APC than for soybean meal (433 vs. 201 g/kg of DM; estimated from INRA, 2007). Accordingly, milk urea concentration was lower for the ER-APC diet compared with the EL diet. This observation seems to confirm the better resistance to ruminal degradation of APC proteins compared with those of soybean meal, as already shown by Lu et al. (1982, 1988). Besides, the higher CP intake with the EL diet may also have contributed to the higher milk urea concentration with this diet compared with the ER-APC diet.

CONCLUSIONS

This experiment shows that corn silage-based diets supplemented with ER as the main UFA source and APC as supplemental 18:3 n-3 source are as effective as corn silage-based diets supplemented with EL as the main UFA and 18:3 n-3 source in increasing bovine milk

UFA and 18:3 n-3 contents. Furthermore, at similar levels of dietary incorporation, ruminal bypass of UFA is higher for APC compared with EL. Subsequently, the transfer efficiency of 18:3 n-3 from diet to milk is higher for APC compared with EL. However, further investigations with multiple levels of 18:3 n-3 intake are necessary to confirm this observation.

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