In Vitro Versus in Situ Ruminal Biohydrogenation of Unsaturated Fatty Acids from a Raw or Extruded Mixture of Ground Canola Seed/Canola Meal

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ABSTRACT

Raw or extruded blends of ground canola seeds and canola meal were used to compare in vitro and in situ lag times and rates of disappearance due to ruminal biohydrogenation of unsaturated fatty acids. The in situ study resulted in higher lag times for biohydrogenation for polyunsaturated fatty acids and lower rates of biohydrogenation of unsaturated fatty acids than the in vitro study, so the in situ biohydrogenation of polyunsaturated fatty acids was not complete at 24 h of incubation. With both methods, rates of biohydrogenation of polyunsaturated fatty acids were higher than for cis-$\triangle$9C18:1. Extrusion did not affect the rate of biohydrogenation of cis-$\triangle$9C18:1, but resulted in higher rates of biohydrogenation of polyunsaturated fatty acids with higher proportions of $trans$ intermediates of biohydrogenation at 4 h of incubation in vitro and at 8 h of incubation in situ. These results suggest that extrusion affects the isomerization of polyunsaturated fatty acids, rather than the hydrogenation steps. In conclusion, in vitro and in situ methods can both show differences of ruminal metabolism of unsaturated fatty acids due to processing, but the methods provide very different estimates of the rates of disappearance due to biohydrogenation.

(**Key words:** biohydrogenation, canola, in situ, in vitro)

Abbreviation key: BH = biohydrogenation, **CLA** = conjugated linoleic acid, **FA** = fatty acid, **RBC** = raw blend of ground canola seeds and canola meal, **EBC** = extruded blend of canola seeds and canola meal, **UFA** = unsaturated FA.

INTRODUCTION

The ruminal digestion of lipids results in major changes of the profile of dietary fatty acids (**FA**) due to biohydrogenation (**BH**) of unsaturated FA (**UFA**). Intermediates of BH, such as conjugated linoleic acid (**CLA**) and $trans$-$\triangle$11C18:1 as a precursor of CLA in the mammary gland of the cow (Griinari et al., 2000) and in human (Adlof et al., 2000) possess interesting dietary properties for human. Increased milk concentration of both CLA and $trans$-C18:1 can be obtained by extrusion of oilseeds (Chouinard et al., 1997a; Bayourthe et al., 2000; Chouinard et al., 2001).

Comparisons of the BH patterns of UFA from unprocessed or processed sources of lipids are usually performed in vivo via comparison between dietary and duodenal flows of FA (Enjalbert et al., 1997; Murphy et al., 1987) or in vitro via the measurement of disappearance of UFA (Harfoot et al., 1973a; Wu and Palmquist, 1991; Beam et al., 2000). In vivo methods are very expensive and time-consuming, and the results obtained in vitro can vary largely as a function of fermentation conditions (Gerson et al., 1983, 1985, 1988; Van Nevel and Demeyer, 1996).

In situ methods have the advantage to incubate substrates in a more physiological milieu than in vitro procedures. They have been widely used for the study of ruminal degradation of protein or fiber but have been infrequently used for the study of ruminal metabolism of lipids (Perrier et al., 1992; Chouinard et al., 1997b).

The objectives of this study were to ascertain the effects of extrusion of canola seeds on lag time and rate of BH of UFA and, using an in vitro study as a reference, to evaluate the accuracy of an in situ procedure for comparison of raw and extruded ground canola.

MATERIALS AND METHODS

Preparation of Canola

Because extrusion of whole fat canola seeds is difficult, a blend of raw canola seeds and canola meal (50/

Table 1. Ingredient and nutrient composition of diets (DM basis).

Ingredients	%
Corn silage	38.4
Ray-grass hay	54.0
Soybean meal	6.8
Mineral-vitamin mix[1]	0.8
Nutrient analysis, g/kg	
CP	11.3
Fat	2.4
ADF	26.6

[1]Contained 5% P, 14% Ca, 6% Na, 4 g of Zn/kg, 3.2 g of Mn/kg, 3 g of Fe/kg, 0.8 g of Cu/kg, 250,800 IU of vitamin A/kg, 62,700 IU of vitamin D3/kg, and 112 IU of vitamin E/kg.

50 wt) were used after grinding through an 8-mm screen. Part of this raw blend of canola (**RBC**) was used to prepare an extruded blend of canola (**EBC**, 140°C exit temperature; Extruder Clextral, BC 45, Firminy, France). Both blends were ground through a 2-mm screen before incubations. The FA composition of RBC and EBC is presented in Table 2.

In Vitro Experiment

The day before each series of incubation, 1.5 g of ground peas as a source of N and starch for fermentation and 4 mg of soybean phosphatidylcholine (Sigma-Aldrich Chimie, Saint Quentin Fallavier Cedex, France) dissolved in 1 ml diethyl ether (Merck, Darmstadt, Germany) to improve lipid dispersion were set into 250-ml flasks. Control cultures and cultures with 800 mg of added RBC or EBC were prepared. Moreover, at 1700 h, nylon bags containing 3.5 g of hay ground through a 5-mm screen were introduced into the rumen of a fistulated dry Holstein cow receiving a diet based on corn silage, hay, and soybean meal (Table 1).

The day of incubation, at 0800 h, nylon bags were removed from the cow, and ruminal fluid was collected, strained through a metal sieve (0.8 mm mesh) and

Table 2. Dry matter and fatty acid composition of raw and extruded blends of ground canola seeds and canola meal.

Treatment	Raw	Extruded
DM, %	90.0	94.0
	(% of DM)	
Total fatty acids	19.0	20.1
Total C18[1]	15.8	16.9
	(% of C18)	
C18:0	1.97	1.90
cis-C18:1[2]	61.63	61.17
cis-C18:2[2]	25.17	25.24
cis-C18:3[2]	11.17	11.69

[1]Fatty acids with 18 carbons.

[2]cis-C18:1 refers to cis-Δ9C18:1, cis-C18:2 refers to cis-Δ9, cis-Δ12C18:2, cis-C18:3 refers to cis-Δ9, cis-Δ12, cis-Δ15C18:3.

brought to the laboratory (30 min). The incubated hay from a nylon bag was added into each flask as a source of fiber and solid-adherent bacteria, because BH takes place on lipids adsorbed onto food particles (Harfoot et al., 1973b). In each flask were added 80 ml of ruminal fluid, and 80 ml of a buffer solution containing (g/L): $NaHCO_3$, 9.24; $Na_2HPO_4.2H_2O$, 19.5; NaCl, 0.705; KCl, 0.675; $CaCl_2.2H_2O$, 0.108, and $MgSO_4.7H_2O$, 0.180, pre-warmed at 39°C, and saturated with CO_2 (pH 6.9).

The flasks were gassed with CO_2 before closing, placed in a water bath rotary shaker (Aquatron; Infors AG, 4103 Bottmingen, Germany) at 39°C, and stirred at 130 rpm. Incubation times for control and canola cultures were 2, 4, 8, 16, and 24 h. Each series of incubation comprised 15 flasks (i.e., for each incubation time, a control culture, a culture with RBC, and a culture with EBC. A supplemental control flask without incubation was kept with each series. Five replicates were incubated for each form of canola and each incubation time. Incubations were stopped by placing the flasks into iced water. Contents of the flasks were then frozen, freeze-dried (Virtis Freezemobile 25; Virtis, Gardiner, NY), weighed, and kept at –18°C until analysis.

In Situ Experiment

Two ruminally-fistulated Holstein dry cows were used. Heat-sealed nylon bags (11×6 cm; mean pore size 45 μm; Blutex, Tissage Tissus Techniques, Combles, France) were filled with 3 g of RBC or EBC. Bags with incubation times of 2, 4, 8, and 24 h were introduced into the rumen before the morning meal, and bags with incubation time of 16 h were introduced before the evening meal. For each incubation time, two replicates were made on each cow. After removal from the rumen, bags were rinsed for approximately 2 min in cold water until no color appeared in the rinse water, frozen at –18°C during 24 h, machine-washed (2×5 min) in cold water, freeze-dried, weighed, and stored at –18°C until analysis. Zero-hour bags were incubated for 1 h in water (38°C), and rinsed, washed, dried, and stored with a similar procedure.

Additionally, the FA composition of material retained in bags incubated in sterilized rumen fluid was investigated to ascertain whether all individual FA had equal losses from the bags. Bags containing RBC or EBC were incubated in 250-ml flasks containing 200 ml of sterilized ruminal fluid and 0.5 g of pancreatin (Sigma Aldrich) in order to hydrolyze triacylglycerols and proteins, because these hydrolyzes, due to microorganisms in the rumen, may affect losses of FA. Two replicates of RBC and EBC were incubated in a water bath rotary shaker during 2, 4, 8, and 16 h. After incubation, the bags were

washed, dried, and stored with the same procedure as bags incubated in situ.

Analysis of Fatty Acids

Samples were ground using a ball mill (Dangoumau, distributed by Prolabo, Nogent-sur-Marne, France). The FA of feed samples and residues of incubation were extracted and methylated with a one-step procedure (Sukhija & Palmquist, 1988). The FA methyl esters were then quantified by GLC (Dani GC 1000, Monza, Italy). The column was a fused silica capillary (CPSil88, $100 \, \text{m} \times .25 \, \text{mm}$ ID, $0.20 \, \mu\text{m}$ film thickness; Chrompack-Varian, Middleburg, the Netherlands). The carrier gas was helium at a constant flow of 1 ml/min. The flame ionization detector was set at 260°C, and the split ratio in the injector (255°C) was 50:1. Oven temperature was initially 50°C and increased by 4°C/min to 200°C, maintained at 200°C for 10 min, increased by 4°C/min to 225°C, maintained at 225°C for 5 min, increased by 5°C/min to 230°C, and maintained at 230°C for 2 min.

With this method, the peaks of $trans$-$\Delta10$C18:1 and $trans$-$\Delta11$C18:1 are difficult to separate one from another, but are separated from peaks of other C18:1 cis or $trans$ isomers, so that FA designated as $trans$-C18:1 in this paper refers to both $trans$-$\Delta10$C18:1 and $trans$-$\Delta11$C18:1. For simplification, FA designated as cis-C18:1, cis-C18:2, and cis-C18:3 will refer to cis-$\Delta9$C18:1, cis-$\Delta9$, cis-$\Delta12$C18:2, and cis-$\Delta9$, cis-$\Delta12$, cis-$\Delta15$C18:3, respectively, and CLA will refer to the cis-$\Delta9$, $trans$-$\Delta11$C18:2 isomer.

Calculations and Statistics

For the in vitro experiment, FA from substrate and inoculum were excluded by subtracting from the FA in cultures with added canola the FA in the control cultures incubated on the same day and during the same time. Such a calculation, already used by others (Beam et al., 2000), assumes that addition of a source of FA does not affect the metabolism of FA from the inoculum or the other substrates.

In both in vitro and in situ experiments, estimation of BH was based on the disappearance of UFA, as in previous studies (Wu and Palmquist, 1991; Reddy et al., 1994; Beam et al., 2000). Estimation of parameters of BH kinetics was based on the exponential model of Orskov and McDonald (1979) and assumed that no immediate disappearance of UFA due to BH could occur, and that all the initial amount of UFA could be hydrogenated.

For the in vitro experiment, no loss of total C18 from the flasks could occur so that at each time of incubation:

$$AC18 = A0C18 \qquad [1]$$

where, AC18 = mg of total C18 at t h of incubation, and A0C18 = mg of total C18 added into the flask. Due to BH, the amount of each UFA remaining in the flasks depended on incubation time as follows:

$$A = A0 \cdot e^{-c \cdot (t - l)} \qquad [2]$$

where, A = mg of the UFA at t h of incubation, A0 = mg of the UFA added into the flask, c = rate of BH, and l = lag time before the BH begins. Dividing the expression [2] by the expression [1], we obtained:

$$P = P0 \cdot e^{-c \cdot (t - l)}$$

where, P = A / AC18 = proportion of the UFA at t h of incubation and P0 = A0 / A0C18 = initial proportion of the UFA in the experimental substrate.

For the in situ experiment, zero-hour bags were used for values at t = 0, and we took into account both disappearance due to physical loss of FA from the bag and disappearance due to BH. Due to loss of FA, the amount of total C18 remaining in the bag after t h of incubation was:

$$AC18 = A0C18 \cdot R \qquad [3]$$

where, AC18 = mg of total C18 at t h of incubation, A0C18 = mg of total C18 at t = 0, and R = proportion of total C18 remaining in the bag a t h of incubation.

Because in this experiment incubation of bags in sterilized rumen fluid showed that all C18 FA had similar losses from the bags and because disappearance of UFA was due both to loss of FA and BH, the amount of each UFA remaining in the bag after t h of incubation was:

$$A = A0 \cdot R \cdot e^{-c \cdot (t - l)} \qquad [4]$$

where, A = mg of the UFA at t h of incubation, A0 = mg of the UFA at t = 0, c = rate of BH, and l = lag time before the BH begins. Dividing the expression [4] by the expression [3], we obtained:

$$P = P0 \cdot e^{(-c \cdot (t-l))}$$

which was the same model as that used for the in vitro experiment.

Data were computed with the nonlinear regression procedure of SYSTAT (Version 9; SPSS Inc., 1998, Chicago, IL). Lag time for BH was considered different from zero when its confidence interval did not contain zero.

Table 3. In vitro experiment: lag time and rate of biohydrogenation of unsaturated fatty acids from canola (mean ± SEM).

Treatment	Raw	Extruded
cis-C18:1[1]		
Lag time, h	−0.43 ± 0.28	−0.11 ± 0.44
Rate of biohydrogenation, %/h	10.4 ± 0.6	11.2 ± 1.1
r^2	0.99	0.97
cis-C18:2[1]		
Lag time, h	−0.01[c] ± 0.17	1.14[a,b] ± 0.18
Rate of biohydrogenation, %/h	19.1[c] ± 1.0	37.5[b] ± 4.2
r^2	0.99	0.97
*cis*C18:3[1]		
Lag time, h	−0.35[c] ± 0.25	1.04[a,b] ± 0.17
Rate of biohydrogenation, %/h	20.4[c] ± 1.6	45.8[b] ± 5.3
r^2	0.98	0.97

[a]Value significantly different from zero ($P < 0.05$).

[b,c]Means in the same row with unlike superscripts differ ($P < 0.01$).

[1]*cis*-C18:1 refers to *cis*-Δ9C18:1, *cis*-C18:2 refers to *cis*-Δ9, *cis*-Δ12C18:2, *cis*-C18:3 refers to *cis*-Δ9, *cis*-Δ12, *cis*-Δ15C18:3.

For the in vitro and the in situ experiments separately, lag times and rates of BH, RBC, and EBC were compared using the model:

$$P = (P0 + \Delta P0\ \text{TRT}) \cdot e^{(-(c + \Delta ce \cdot \text{TRT})\ (t-(l + \Delta le \cdot \text{TRT})))}$$

where, $\Delta P0$ = difference of proportion of each FA between RBC and EBC, TRT is coded 0 for RBC and 1 for EBC, so that Δce and Δle are the effects of extrusion on rate and lag time of BH, respectively. The effects of extrusion were declared significant when the confidence interval of Δce and Δle did not contain zero.

The effects of method of investigation and interaction of extrusion by method of investigation were tested using the model:

$$A = (P0 + \Delta P0\ \text{TRT}) \cdot e^{(-(c + \Delta ce \cdot \text{TRT} + \Delta cm \cdot \text{MODE} + \Delta cem \cdot \text{TRT} \cdot \text{MODE})\ (t-(l + \Delta le \cdot \text{TRT} + \Delta lm \cdot \text{MODE} + \Delta lem \cdot \text{TRT} \cdot \text{MODE})))}$$

where, MODE is coded 0 for the in vitro experiment and 1 for the in situ experiment, so that Δcm and Δlm are the effects of method on rate and lag time of BH, respectively, and Δcem and Δlem are the effects of interaction of extrusion by method on rate and lag time of BH, respectively.

At each time of incubation, the proportions of each FA were compared between RBC and EBC for the in vitro and the in situ experiments separately, using the general linear model of SYSTAT.

RESULTS

In the in vitro experiment, most lag times for BH of the UFA were not significantly different from zero, except lag times for BH of *cis*-C18:2 and *cis*-C18:3 from EBC (Table 3). The rate of BH was twice as high for *cis*-C18:2 and *cis*-C18:3 than for *cis*-C18:1 with RBC, and was higher after extrusion for *cis*-C18:2 and *cis*-

Table 4. In vitro experiment: effects of extrusion and time of incubation on the profile of fatty acids with 18 carbons from canola.

Treatment	Time of incubation, h														
	2			4			8			16			24		
	RBC[1]	EBC[2]	SEM	RBC	EBC	SEM	RBC	EBC	SEM	RBC	EBC	SEM	RBC	EBC	SEM
	(% of C18)														
C18:0	16.48	11.13	3.19	33.18[b]	38.12[a]	1.24	51.87	53.06	1.99	70.36	67.1	1.84	76.87	72.98	1.59
cis-C18:1[3]	49.08	52.63	2.22	37.29[a]	33.65[b]	1.10	23.77	21.71	1.20	11.33	11.24	0.74	8.63	9.56	1.04
trans-C18:1[3]	5.89	4.15	0.54	8.20[d]	12.36[c]	0.59	11.76[b]	15.18[a]	0.73	11.16[b]	13.65[a]	0.74	10.78	11.83	0.75
cis-C18:2[3]	17.03	18.67	1.01	11.42[c]	7.53[d]	0.62	5.53[c]	3.41[d]	0.44	1.67	1.70	0.16	0.77	1.25	0.20
CLA[3]	−0.28	0.47	0.39	0.08[d]	0.36[c]	0.05	0.31	0.26	0.06	0.09	0.14	0.04	0.02	0.12	0.08
cis-C18:3[3]	6.66	7.62	0.51	4.56[c]	2.76[d]	0.25	2.27[c]	1.00[d]	0.26	0.55	0.64	0.13	0.09	0.51	0.17

[a,b,c,d]Means in the same row and for the same incubation time with unlike superscripts differ (a, b, $P < 0.05$; c, d, $P < 0.01$).

[1]RBC: raw blend of ground canola seeds and canola meal.

[2]EBC: extruded blend of canola seeds and canola meal.

[3]*cis*-C18:1 refers to *cis*-Δ9C18:1, *trans*-C18:1 refers to *trans*-Δ10C18:1+ *trans*-Δ11C18:1, *cis*-C18:2 refers to *cis*-Δ9, *cis*-Δ12C18:2, CLA refers to *cis*-Δ9, *trans*-Δ11C18:2, *cis*-C18:3 refers to *cis*-Δ9, *cis*-Δ12, *cis*-Δ15C18:3.

Figure 2. In situ experiment: disappearance of fatty acids with 18 carbons from canola. Error bars are ± SE.
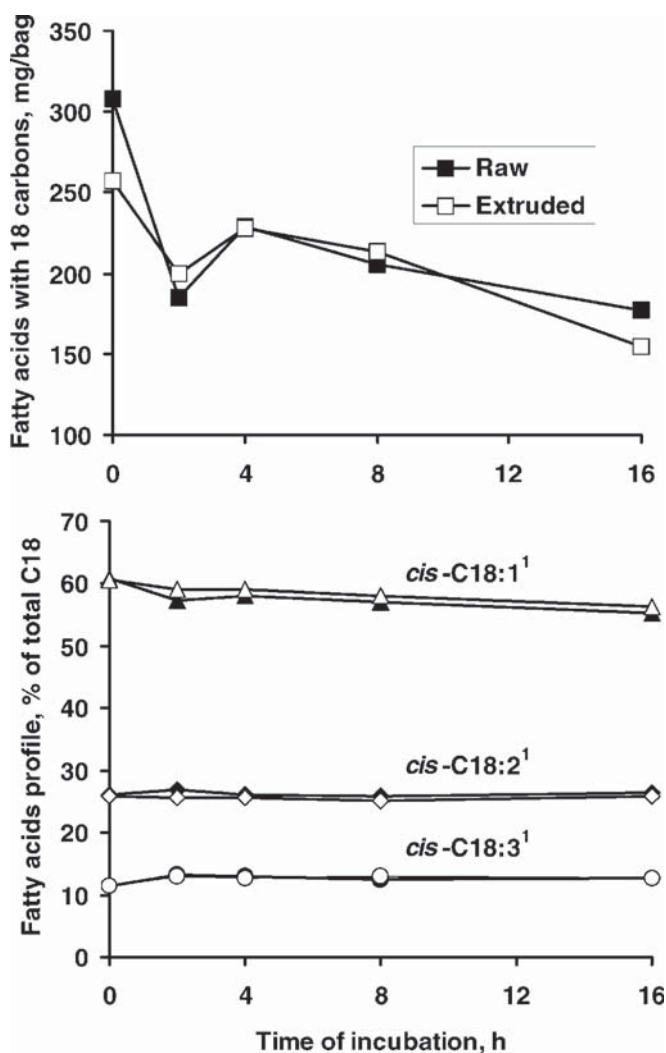
Figure 1. Effects of incubation of bags containing canola in sterilized rumen fluid with added pancreatin on disappearance of fatty acids with 18 carbons and evolution of their profile. [1]*cis*-C18:1 refers to *cis*-Δ9C18:1, *cis*-C18:2 refers to *cis*-Δ9, *cis*-Δ12C18:2, *cis*-C18:3 refers to *cis*-Δ9, *cis*-Δ12, *cis*-Δ15C18:3.

C18:3. The proportions of *cis*-C18:1, *cis*-C18:2, and *cis*-C18:3 at 4 h of incubation, and of *cis*-C18:2 and *cis*-C18:3 at 8 h of incubation, were lower with EBC (Table 4). By contrast, at 4 h of incubation, proportions of C18:0, *trans*-C18:1, and CLA were higher with EBC, and *trans*-C18:1 remained higher with EBC at 8 and 16 h of incubation. The proportion of *trans*-C18:1 decreased very slowly from 8 h of incubation, so that *trans*-C18:1 was the most abundant UFA at 24 h of incubation.

The disappearance and the profile of FA in bags incubated in sterilized ruminal fluid with added pancreatin are shown on Figure 1. The profiles of FA were similar
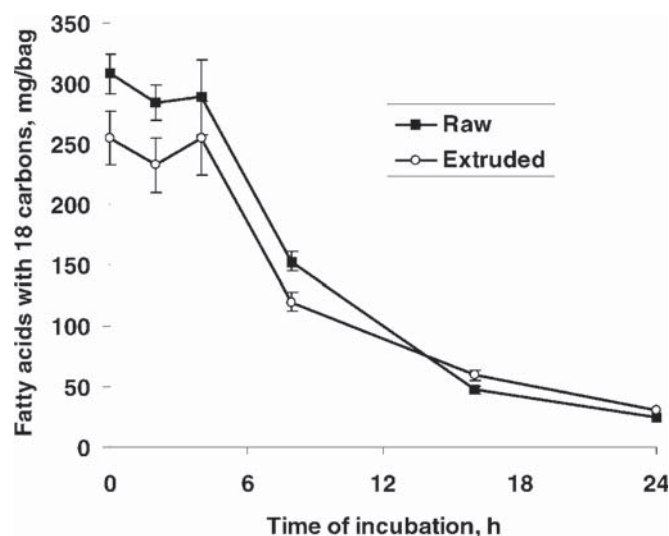
to the profile of FA in RBC or EBC for all incubation times, showing that the rate of loss through the bag was similar for all FA. In the in situ experiment, disappearance of total C18 through the bag was rapid after 4 h of incubation (Figure 2), so that only about 30 mg of total C18 remained in the bag at 24 h of incubation. Lag times for BH of UFA were significantly greater than zero with EBC (Table 5). Lag times for BH of *cis*-C18:2 and *cis*-C18:3 were significantly higher than in the in vitro experiment (Table 6) but were not affected by extrusion. Extrusion resulted in higher rates of BH of *cis*-C18:2 and *cis*-C18:3 (Table 5). Rates of BH of all UFA were on average four times as low as in the in vitro experiment. This difference was highly significant, and no interaction of method of investigation by extrusion was observed (Table 6). Extrusion resulted in lower proportions of *cis*-C18:2 and *cis*-C18:3 and higher proportions of C18:0, *trans*-C18:1 and CLA at 8 h of incubation (Table 7). Proportions of *cis*-C18:2 and *cis*-C18:3 remained numerically lower at 16 h of incubation and significantly lower at 24 h of incubation with EBC.

DISCUSSION

Effect of the Method of Investigation on the Kinetics of Biohydrogenation of Fatty Acids from Canola

A lag time has not been described in previous in vitro kinetics studies when *cis*-C18:2 (Harfoot et al., 1973a; Kellens et al., 1986; Beam et al., 2000) or soybean oil (Beam et al., 2000) were added as a lipid source. In situ lag times for BH of UFA were between 1 and 2 h, so

Table 5. In situ experiment: lag time and rate of biohydrogenation of unsaturated fatty acids from canola (mean ± SEM).

Treatment	Raw	Extruded
cis-C18:1[1]		
Lag time, h	1.06 ± 1.03	1.69[a] ± 0.67
Rate of biohydrogenation, %/h	2.6 ± 0.3	3.4 ± 0.2
r[2]	0.99	0.99
cis-C18:2[1]		
Lag time, h	1.90 ± 1.05	1.84[a] ± 0.33
Rate of biohydrogenation, %/h	4.8[c] ± 0.7	10.0[b] ± 0.8
r[2]	0.99	0.99
cis-C18:3[1]		
Lag time, h	1.85 ± 1.10	1.69[a] ± 0.33
Rate of biohydrogenation, %/h	4.9[c] ± 0.8	12.5[b] ± 1.2
r[2]	0.97	0.98

[a]Value significantly different from zero ($P < 0.05$).

[b,c]Means in the same row with unlike superscripts differ ($P < 0.01$). [1]*cis*-C18:1 refers to *cis*-Δ9C18:1, *cis*-C18:2 refers to *cis*-Δ9, *cis*-Δ12C18:2, *cis*-C18:3 refers to *cis*-Δ9, *cis*-Δ12, *cis*-Δ15C18:3.

the FA profile at 2 h of incubation was similar to that of canola blends. Perrier et al. (1992), measuring total in situ disappearance of individual FA due both to loss of particles and to BH reported, at 4 h of incubation, disappearances around 40 and 42% for total FA and *cis*-C18:2, respectively, which suggests that BH of *cis*-C18:2 was very limited during the first 4 h incubation and was consistent with an important lag time. This could be due to a lag time for lipolysis, because ruminal BH can only occur on FFA, not on FA in triacylglycerols (Hawke and Silcock, 1969). Previous studies have shown that lipolysis of triacylglycerols is very rapid in the rumen (Garton et al., 1958; Beam et al., 2000), and such an hypothesis would not explain the difference between the in situ and the in vitro experiments. Such a lag time has often been reported for the degradation of fiber, due to time for colonization of the bags and adhesion of cellulolytic bacteria on cell walls. The BH is known to occur on FA adsorbed onto feed particles (Harfoot et al., 1973b). One of the most important bacteria for BH is a cellulolytic bacterium, *Butyrivibrio fibri-*

Table 6. Significance of effects of method of investigation and interaction of method of investigation by extrusion on lag time and rate of biohydrogenation of unsaturated fatty acids from canola.

	Method of investigation	Interaction method × extrusion
cis-C18:1[1]		
Lag time	NS	NS
Rate of biohydrogenation	$P < 0.01$	NS
cis-C18:2[1]		
Lag time	$P < 0.05$	NS
Rate of biohydrogenation	$P < 0.01$	NS
cis-C18:3[1]		
Lag time	$P < 0.05$	NS
Rate of biohydrogenation	$P < 0.01$	NS

[1]*cis*-C18:1 refers to *cis*-Δ9C18:1, *cis*-C18:2 refers to *cis*-Δ9, *cis*-Δ12C18:2, *cis*-C18:3 refers to *cis*-Δ9, *cis*-Δ12, *cis*-Δ15C18:3.

solvens (Polan et al., 1964), so that the BH of FA could require a similar lag time than degradation of fiber in situ. By contrast, in this in vitro experiment hay was incubated in the rumen and provided cellulolytic bacteria already attached onto particles. Therefore, stirring the flask could result in a rapid adsorption of lipids onto hay particles, resulting in lower lag times for BH.

Comparing in vitro batch cultures and in situ methods for the study of NDF degradation, Varel and Kreikemeier (1995) obtained lower lag times in situ than in vitro. The opposite results on UFA in this experiment could be due 1) to intrinsic differences between lag times for fibrolysis and BH by bacteria or 2) to differences of colonization pattern between main cellulolytic bacteria and bacteria that are both cellulolytic and responsible for BH, or 3) to a slow penetration of bacteria into the nylon bag because of hydrophobicity of canola, contrasting with a rapid dispersion of the lipids in the in vitro experiment.

The BH of UFA was rapid in this in vitro experiment. Previous in vitro studies have often focused on the BH of *cis*-C18:2 and resulted in contrasting results. Reddy et al. (1994) found that BH of *cis*-C18:2 from raw soybeans was 79.8% at 24 h of incubation when calculated with the proportion of *cis*-C18:2 in total C18, which means that the ratio *cis*-C18:2/total C18 was five times as low at 24 h than initially. In this experiment, this ratio was 30 times as low than initially at 24 h. Beam et al. (2000) also published slower rates of BH of *cis*-C18:2, ranging from 8 to 12%/h. By contrast, Wu and Palmquist (1991) found that the in vitro BH of *cis*-C18:2 from an animal-vegetable fat blend was about 90% at only 4 h of incubation, which implies a rate of BH around 40%. Van Nevel and Demeyer (1996) published BH over 90% for *cis*-C18:2 from soybean oil at 6 h of incubation. Similarly, conflicting results have been pub-

Table 7. In situ experiment: effects of extrusion and time of incubation on the profile of fatty acids with 18 carbons from canola.

	Time of incubation, h																	
	0^1			2			4			8			16			24		
Treatment	RBC^2	EBC^3	SEM	RBC	EBC	SEM	RBC	EBC	SEM	RBC	EBC	SEM	RBC	EBC	SEM	RBC	EBC	SEM
							(% of C18)											
C18:0	1.91	1.97	0.08	2.55	2.80	0.48	4.48	5.26	1.00	6.31[d]	11.41[c]	0.90	18.45	23.77	3.07	24.28	31.06	5.70
cis-C18:1[4]	60.78	60.61	0.29	60.09	60.23	0.26	55.16	55.27	0.56	55.29	53.43	0.72	38.82	33.16	2.09	34.74	30.89	3.49
transC18:1[4]	0.00	0.00		0.59	0.67	0.21	2.61	2.94	0.78	6.05[b]	12.23[a]	1.56	21.08	29.34	2.74	23.90	31.02	2.75
cis-C18:2[4]	26.08	25.78	0.14	25.15	24.65	0.37	21.63	20.66	0.89	21.37[a]	13.70[b]	1.74	10.02	5.22	2.16	10.24[a]	3.68[b]	1.25
CLA[4]	0.00	0.00		0.17	0.23	0.07	0.37	0.44	0.16	0.91[b]	2.74[a]	0.38	0.36	0.38	0.03	0.23[b]	0.65[a]	0.10
cis-C18:3[4]	11.40	11.46	0.27	11.27	11.24	0.16	9.61	8.99	0.55	9.07[c]	4.80[d]	0.80	4.23	1.97	0.97	4.72[a]	1.42[b]	0.68

[a,b,c,d]Means in the same row and for the same incubation time with unlike superscripts differ (a, b, $P < 0.05$; c, d, $P < 0.01$).

[1]Incubated 1 h in water and washed.

[2]RBC: raw blend of ground canola seeds and canola meal.

[3]EBC: extruded blend of canola seeds and canola meal.

[4]cis-C18:1 refers to cis-$\Delta9$C18:1; trans-C18:1 refers to trans-$\Delta10$C18:1+ trans-$\Delta11$C18:1, cis-C18:2 refers to cis-$\Delta9$, trans-$\Delta11$C18:2, CLA refers to cis-$\Delta9$, trans-$\Delta11$C18:2, cis-C18:3 refers to cis-$\Delta9$, cis-$\Delta12$, cis-$\Delta15$C18:3.

lished about BH of cis-C18:1, with an extent of BH between 50 and 60% at 4 h of incubation (Wu and Palmquist, 1991), or a rate of approximately 3.6 %/h (Beam et al., 2000). Such large variations among experiments, including ours, can be related to experimental conditions, because in vitro BH depends on the starch content of the substrate (Gerson et al., 1985), the N content (Gerson et al., 1983), the pH (Van Nevel and Demeyer, 1996), and the size of particles (Gerson et al., 1988). Ruminal BH of cis-C18:3 in vitro has been reported to be in the same range as BH of cis-C18:2 (Wu and Palmquist, 1991), which is consistent with results of the current experiment.

Depending on the source and amount of added unprotected fat, BH has been reported to be in the range 60 to 73%, 75 to 87%, and 83 to 88% in vivo for cis-C18:1, cis-C18:2, and cis-C18:3 (Murphy et al., 1987; Wu et al, 1991). In vitro calculations from BH kinetic parameters reported in Table 3 indicate that these ranges of BH were attained between 8 and 12 h of incubation with RBC. By contrast, such BH was not yet attained at 24 h of incubation in situ, because of much lower rates of BH.

Perrier et al. (1992) reported, in an in situ experiment with ground soybeans as a substrate, that at 8 h of incubation disappearances were about 60 and 75% for total FA and cis-C18:2, respectively. From these results, it can be calculated that the percentage of cis-C18:2 in total FA, which was initially 50%, decreased to about 30% at 8 h of incubation, which suggests a slow BH. In this experiment, using BH kinetic parameters reported in Table 5, it can be calculated that a similar 40% decrease of the proportion of cis-C18:2 with RBC was reached at 13 h of incubation. A slow penetration of bacteria into the bag, or the lack of fibrous particles in the bag, could also explain the lower rate of BH observed in situ than in vitro, contrasting with higher degradation rates of fiber observed by Varel and Kreikemeier (1995), but consistent with lower degradation rates of proteins observed by Broderick et al. (1988). Under this hypothesis, mixing a source of fiber with the source of fat in the bags could hasten both adsorption of lipids and adhesion of bacteria onto particles, resulting in lower lag times and higher rates of BH.

An important accumulation of trans-C18:1 was observed, especially in situ. At 24 h of incubation, trans-C18:1 represented 23.9% of total C18 remaining in the bags with RBC, and from the beginning of incubation, the percentages of cis-C18:2 plus cis-C18:3, which both are precursors of trans-C18:1 (Harfoot and Hazlewood, 1988), had decreased from 37.5 to 15.0%, which means that nearly all the cis-C18:2 and cis-C18:3 that disappeared were transformed into trans-C18:1, not C18:0. Consistently, at 24 h of incubation, the increase of C18:0

and the decrease of *cis*-C18:1 proportions were similar. Accumulation of *trans*-C18:1 as the major end-product of BH of *cis*-C18:2 has been described in vitro (Wu and Palmquist, 1991), and when the initial concentration of *cis*-C18:2 is over 1 mg/ml, the inhibition of BH of *trans*-C18:1 to C18:0 is irreversible (Harfoot et al., 1973a). In this in vitro experiment, the initial concentration of *cis*-C18:2, including *cis*-C18:2 from substrate and inoculum, was about 0.28 mg/ml, so that *trans*-C18:1 did not accumulate as a major end-product. In the in situ experiment, the initial concentration of *cis*-C18:2 in the bags was 36 mg/g, which probably resulted in concentrations of *cis*-C18:2 largely over 1 mg/ml after penetration of ruminal fluid into the bags.

Effect of the Method of Investigation on the Modifications of Biohydrogenation Due to Extrusion

Both in vitro and in situ, the rates of BH of *cis*-C18:2 and *cis*-C18:3 were much higher with EBC. This could be due to the rupture of cell membranes, with an increased availability of free oil for ruminal fermentation (Reddy et al., 1994). In their experiment, Reddy et al. (1994) found in vitro that extrusion of soybeans lowered the extent of BH, but with an initial concentration of *cis*-C18:2 that was much higher than in this experiment, which could have affected lipolysis and BH (Beam et al., 2000).

This faster BH with EBC resulted in lower proportions of UFA at 4 and 8 h of incubation in vitro and 8 h of incubation in situ. However, this faster disappearance seemed to be related mainly to the first step of ruminal BH (i.e., isomerization) because extrusion also resulted in higher concentrations of CLA and *trans*-C18:1. The ratios of CLA to disappeared *cis*-C18:2 and of *trans*-C18:1 to disappeared (*cis*-C18:2 + *cis*-C18:3) were similar with RBC and EBC, suggesting that rates of BH of these intermediates were not affected by extrusion. Lack of effect of extrusion on the rate of BH of *cis*-C18:1 is consistent with an effect of extrusion on the isomerization of polyunsaturated FA because BH of *cis*-C18:1 does not require the action of an isomerase.

Comparing raw and extruded soybeans in situ, Chouinard et al. (1997b) found that the concentrations of all C18 FA in the bags were higher with extruded soybeans and outlined that these results were consistent with higher *trans*-C18:1 proportions in plasma and milk, but contrasted with lower *cis*-C18:2 and *cis*-C18:3 proportions in milk. In this experiment, extrusion resulted, both in vitro and in situ, in higher proportions of *trans*-C18:1 and CLA and lower proportions of *cis*-C18:2 and *cis*-C18:3, which is consistent with the variations of milk fat composition observed when extruded soybeans are compared to raw ground soybeans

(Chouinard et al., 1997a, 1997b). The differences between these in situ results and the results published by Chouinard et al. (1997b) are due to differences in the method of expression of FA disappearance, because calculations of these authors did not separate disappearance due to loss of particles and disappearance due to BH.

Compared to a control diet without added fat, the increase of proportion of *trans*-C18:1 in milk is much higher with added extruded canola than with added raw ground canola (Bayourthe et al., 2000), which is consistent with the increased peak proportions of *trans*-C18:1 observed in these experiments. Similarly, with EBC, the peak proportion of CLA was 4 and 3 times as high as with RBC in vitro and in situ, respectively. Such ratios are consistent with the threefold greater concentration of CLA in milk from cows receiving extruded soybeans compared with cows receiving raw ground soybeans (Chouinard et al., 2001), which could also have resulted from a mammary desaturation of *trans*-C18:1 (Griinari et al., 2000).

CONCLUSIONS

Extrusion of canola increased the rate of BH of UFA and the proportions of intermediates of BH that fit with the concern of increasing the concentration of CLA in milk and milk products. In vitro and in situ studies resulted in different estimates of the rates of BH of UFA, but resulted in similar differences between RBC and EBC. In conclusion, both methods can be used to study differences of rates of BH due to treatment of oilseeds. More research is needed to ascertain whether a slow microbial colonization or a high initial concentration of *cis*-C18:2 in the in situ method can explain the low BH rates observed with this method.

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