



Influence of extrusion of white lupines on ruminal biohydrogenation of polyunsaturated fatty acids and milk fat composition in dairy cows

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ABSTRACT

The objective was to evaluate the effects of extrusion of white lupines, with or without addition of reducing sugars before extrusion, on the ruminal biohydrogenation (RBH) of dietary PUFA, their transfer rates into milk, and the milk fatty acid (FA) profile in dairy cows. Sixteen multiparous Holstein cows were assigned to 2 separate replicated 4 × 4 Latin square design experiments (8 cows in each experiment). Diets contained 20% (DM basis) raw or extruded whole white lupines (*Lupinus albus*) processed at 115°C, 135°C, or 150°C, and either without (experiment 1 [Exp.1]) or with (experiment 2 [Exp.2]) the addition of reducing sugars. All diets had a forage/concentrate ratio of 69:31 and were isoenergetic, isonitrogenous, and iso-starch. Experiment 1 included 3 rumen-cannulated cows to assess RBH by analyzing ruminal FA profiles 5 h and 17 h after feeding. Ruminal CH₄ production was measured with an automated head-chamber system (excluding rumen-cannulated cows, n = 4 in Exp.1, and n = 8 in Exp.2). Extrusion increased the proportion of *trans* MUFA and CLA in milk fat, with the greatest effects observed at 150°C, irrespective of sugar addition. Milk from cows fed lupines extruded at 150°C contained higher proportions of n-3 and n-6 PUFA, compared with all other dietary treatments. The RBH of *trans*-11 C18:1 and *cis*-9,*trans*-11 C18:2 was also higher with lupines extruded at 150°C than with raw lupines. Extrusion and temperature of extrusion did not affect CH₄ production (g CH₄/d). However, CH₄ intensity (g CH₄/kg ECM) was reduced by 11% on average with lupines extruded at 150°C without addition of reducing sugars compared with raw lupines and lupines extruded at 115°C and 135°C. In conclusion, extrusion of lupines, particularly at 150°C, altered RBH, and increased the

proportions of *trans* MUFA and PUFA in milk fat, but did not reduce CH₄ production in dairy cows.

Key words: white lupine, thermomechanical treatment, fatty acids, biohydrogenation, dairy cow

INTRODUCTION

Interest in alternative leguminous seeds such as lupines is increasing in Europe because of their potential as indigenous protein sources for dairy cow diets. White lupine (*Lupinus albus*) seeds contain, on average, 9.6% ether extract (EE) in DM, with reported values ranging from 4.2% to 14.3% (INRAE-CIRAD-AFZ, 2025), which is higher than in field peas (0.6%–2.6% EE in DM) but much lower than in soybeans (21.5% EE in DM according to INRAE-CIRAD-AFZ, 2025). The fatty acid (FA) profile of white lupines is dominated by oleic acid (49.1% of total FA), linoleic acid (LA; 14.7%), α-linolenic acid (ALA; 8.3%), and palmitic acid (7.4%).

In the feed processing, thermomechanical treatments such as extrusion are widely applied to leguminous seeds to reduce ruminal CP degradability and increase the supply of RUP. The inclusion of reducing sugars before extrusion has been explored in lupines and fava beans to enhance heat treatment efficiency via Maillard reactions (Mendowski et al., 2020). Recent work demonstrated that extrusion of white lupines at 150°C, without sugar addition, reduced ruminal CP degradability and improved milk yield and nitrogen use efficiency in dairy cows (Manzocchi et al., 2023).

Beyond protein, extrusion also affects the lipid fraction of seeds. By disrupting plant cells structures, extrusion can release cytosolic triglycerides, increase emulsification, reduce particle size, and enhance access to ruminal lipases, isomerases, and hydrogenases, thereby modifying ruminal biohydrogenation (RBH) of FA (Doreau et al., 2009; Troegeler-Meynadier et al., 2014). Extrusion may additionally induce interactions between lipids and starch, polysaccharides, or proteins (Hülsebusch et al.,

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-26. Nonstandard abbreviations are available in the Notes.

2025), and depending on the processing conditions, can degrade or liberate antioxidants (e.g., tocopherols) or generate new antioxidant molecules such as Maillard reaction compounds (Mendowski et al., 2019). Thus, extrusion temperature and the presence of reducing sugars are expected to influence the stability, availability, and ruminal fate of dietary lipids.

Extrusion effects on lipid metabolism have been extensively studied in linseed and soybeans. For example, extruded linseed increased ruminal disappearance of ALA and duodenal flow of MUFA (Gonthier et al., 2004) and led to higher milk *trans* MUFA due to altered RBH (Doreau et al., 2009). Other studies found no changes in duodenal FA composition but increased RBH intermediates in milk fat (Akraim et al., 2007). Similarly, extrusion of soybeans reduced RBH of PUFA and altered their availability for absorption (Chouinard et al., 1997).

Dietary lipids can also mitigate CH₄ emissions. Increasing dietary lipids via full-fat oilseeds or oils typically reduces CH₄ production by 1% to 7% per 10 g of added lipids per kilogram of DM (Eugène et al., 2008; Martin et al., 2020a), with mitigation depending on both lipid concentration and accessibility within the feed matrix (Martin et al., 2020a). Research on CH₄ mitigation through extrusion has focused primarily on linseed, which contains higher EE and ALA concentrations than lupines (Martin et al., 2008, 2010; Livingstone et al., 2015). In these studies, extruded linseed reduced CH₄ production more effectively than raw linseed, with a reduction of up to 38% (included at 21.5% in the dietary DM) in comparison to a control corn silage-based diet including linseed meal (Martin et al., 2008).

In contrast, much less is known about the effects of lupine extrusion on ruminal lipid metabolism and CH₄ emissions. Staerfl et al. (2012) evaluated raw white lupines in fattening bulls, whereas Bryszak et al. (2020) investigated narrow-leaved blue lupines (*Lupinus angustifolius*) as a partial replacer of rapeseed and soybean meal in dairy cows. To our knowledge, no study has examined how thermomechanical processing of white lupines influences the RBH of PUFA, milk FA profile, and enteric CH₄ production.

Building on our previous work (Manzocchi et al., 2023), which showed that white lupine extrusion at 150°C reduced ruminal CP degradability and improved milk yield and N use efficiency, likely via Maillard reaction compounds and protein matrix modifications, we now address the effect of the same processing factors on lipid metabolism. Two experiments were conducted to evaluate the effects of extruding white lupines at 3 temperatures (115°C, 135°C, 150°C). In experiment 1 (**Exp.1**), lupines were extruded without the addition of reducing sugars, whereas in experiment 2 (**Exp.2**), reducing sugars were added before extrusion. The objectives were to

assess the effects of these treatments on (1) the apparent postprandial variation of ruminal C18 FA proportions as indicators of RBH, (2) the milk FA profile, and (3) the transfer rate of PUFA to milk. In the present study, we hypothesized that extrusion, at higher temperatures and potentially amplified by reducing sugars, would alter FA accessibility to rumen microbes, leading to a partial inhibition of RBH, greater accumulation and outflow of RBH intermediates (e.g., vaccenic acid and CLA precursors), and increased transfer of PUFA and intermediates into milk, while also exerting limited effects on enteric CH₄ at the tested lipid inclusion level.

MATERIALS AND METHODS

The experiments were approved by the ethics committee of the Auvergne-Rhône-Alpes region and the French Ministry of Higher Education, Research, and Innovation (approval APAFIS#15401-2017062616304407 v5) and conducted at the INRAE Herbipôle research facility (Theix, France) from November 2020 to March 2021. Data on the effects of extrusion and extrusion temperature of white lupines on N use efficiency, N partitioning, and Nε-carboxymethyl-lysine were obtained from the same experiment and have been previously reported (Manzocchi et al., 2023). That publication also provides detailed information on nutrient digestibility, ruminal fermentation characteristics (VFA profile and ammonia concentration), and the kinetics of ruminal parameters measured in rumen-cannulated cows.

Experimental Design, Animals, and Diets

The design of the 2 experiments and the experimental diets have been described in detail by Manzocchi et al. (2023). Two separate replicated 4 × 4 Latin square experiments were conducted simultaneously using a total of 16 multiparous Holstein cows. Each experiment enrolled 8 different cows, and all 16 cows were housed together in a single pen in a freestall barn with regulated access to individual feeding troughs, except during d 21 to 27 of each period, when 8 cows (4 per experiment) were moved to tiestalls for digestibility and nitrogen balance measurements (Manzocchi et al., 2023). The cows had been previously accustomed to individual stalls, and no obvious behavioral changes were observed. Cows included in Exp.1 averaged 3.0 ± 0.4 (mean ± SE) lactations, 91 ± 34 DIM, 25 ± 1.5 kg milk/d, and 704 ± 23.9 kg BW at the start of the experiment, whereas cows included in Exp.2 averaged 2.8 ± 0.3 lactations, 57 ± 4 DIM, 25 ± 1.5 kg milk/d, and 667 ± 16.6 kg BW. Each experiment included 4 dietary treatments tested over four 28-d periods, with the first 14 d of each period used for dietary adaptation followed by 14 d of measurements; treatment order was

balanced to minimize carryover effects. Although the Latin square design inherently controls variation due to animal and period, treatment allocation within each square was randomized to reduce the risk of systematic bias and to satisfy assumptions of independence. In Exp.1, cows received TMR diets containing raw white lupine (*Lupinus albus*) seeds (RL), or the same seeds extruded at 115°C (EL1), 135°C (EL2), or 150°C (EL3). In Exp.2, reducing sugars (1.25% of dextrose monohydrate) were added to lupines before extrusion and were tested either raw (RLS) or extruded at 115°C (ELS1), 135°C (ELS2), or 150°C (ELS3). Details of the sugar pretreatment and extrusion process are provided in Manzocchi et al. (2023). Experimental lupines were dried at 60°C for 72 h and then sieved at 1 mm to determine the particle fraction at >1 mm and <1 mm.

Among the 16 cows, 3 were rumen-cannulated (3.7 ± 0.7 lactations, 167 ± 76 DIM, 22.1 ± 1.9 kg milk/d, 748 ± 3.7 kg BW) and all participated in Exp.1. The TMR consisted of (DM basis) 30% corn silage, 24% grass silage (first cut of semi-mountainous grassland with *Dactylis glomerata*, *Arrhenatherum elatius*, and *Lolium perenne* as the main botanical species), 15% hay (first cut of semi-mountainous grassland with *Alopecurus pratensis*, *Holcus lanatus*, *Arrhenatherum elatius*, and *Lolium perenne* as the main botanical species), 11% pelleted concentrate, and 20% lupines (raw or extruded, depending on the treatment). Lupines were thoroughly mixed into the TMR. All cows also received 200 g/d of a vitaminized-mineral feed (Galaphos MiDi Duo, CCPA) and 20 g/d of rumen-protected DL-methionine (Smartamine M, Adisseo). Diets were formulated to be isoenergetic (NEL expressed in Unité Fourragère Lait [UFL], where 1 UFL = 7.37 MJ), isonitrogenous (15.4% CP in the DM), iso-starch (16% starch in the DM), and iso-NDF (40% NDF in the DM). The TMR was offered twice daily (0900 and 1600 h), and diet transitions between periods were managed over 3 d by replacing 25% of the previous lupine source daily. Cows had free access to water and NaCl blocks and were milked twice daily at 0800 and 1530 h in a herringbone milking parlor, with individual milk yield recorded automatically (DeLaval milk meter MM27BC, DeLaval, France). Cows kept in the tiestalls were milked with a milking pipeline, and individual milk yield was recorded automatically. All samples of feed, milk, blood, and ruminal digesta were blinded with respect to dietary treatment before laboratory processing.

Measurements, Samplings, and Laboratory Analyses

Feed Intake and Diet Composition. Raw and extruded lupines, grass silage, corn silage, hay, pelleted concentrate, and the TMR (200 g each) were sampled on d 21, 24, and 26 of each experimental period, pooled by pe-

riod, and preserved either at -20°C (lupines, grass and corn silages) or at ambient temperature in the dark (hay). Raw and extruded lupines and pelleted concentrate were further pooled across the entire experiment, lyophilized, and ground to pass a 1-mm screen before analysis. Forages were lyophilized, ground (1-mm screen), and pooled to obtain one sample per period. Dry matter intake was determined by recording feed offered and individualorts. Dry matter concentration of the grass and corn silages was determined 3 times per week during wk 1 to 4 of each experimental period. The DM of the lupines and the pelleted concentrate feed was determined once per week during wk 1 to 4. The DM content of the basal mixed diet was determined twice per week during wk 1 to 3 and daily from d 21 to d 27 of each experimental period. Orts were sampled and analyzed for DM whenever they exceeded 5% of the offered ration. Pelleted concentrate provided as bait feed in the automated head-chamber (AHC) system was accounted for in total DMI by multiplying the number of bait feed drops per visit by 40 g/drop and by the DM content of the bait feed, assuming full consumption. Chemical composition of feeds was analyzed as described by Manzocchi et al. (2023). Ether extract of lupines, corn silage, grass silage, hay, and concentrate was analyzed gravimetrically after acid hydrolysis and ether extraction (ISO 6492:1999; ISO, 1999). Gross energy (GE) of lupines and the basal mixed ration was measured with a combustion calorimeter (model C200, IKA, Staufen, Germany). Fatty acid profiles of feeds were analyzed by GC following lipid extraction with chloroform:methanol (1:3), methylation with methanolic HCl, and addition of an internal standard (C23:0, tricosanoic acid), as described by Ferlay et al. (2010). Individual cow data on DMI were averaged over wk 3 and 4 of each period. Fatty acid intake was calculated as the FA concentration in dietary DM multiplied by individual DMI. The DMI data from one cow (treatment EL3, period 1) were excluded because of aberrant values due to lameness.

Milk. Individual milk samples were collected on d 20, 24, 26, and 27 at the morning and evening milking and preserved with 2-bromo-2-nitropropane-1,3-diol for analysis of fat, true protein, lactose, and urea by Fourier-transform mid-infrared spectroscopy (AgroLab's, Aurillac, France). Milk samples of cows milked in the herringbone milking parlor were sampled with an in-line automatic milk sampler, whereas milk of cows kept in metabolic stalls was sampled with a portable milk meter. Milk yield and composition were averaged over 4 sampling days of each experimental period, and the ECM yield (kg/d) was calculated according to Madsen et al. (2008) as follows: $[23.8 \times \text{protein yield (kg/d)} + 38.9 \times \text{fat yield (kg/d)} + 16.3 \times \text{lactose yield (kg/d)}]/3.14$. On d 27 of each period, individual milk samples (3 mL) were collected from 2 consecutive milkings and stored at

−20°C, lyophilized (Thermovac TM-20, Froilabo), and pooled per day according to milk fat yield of each milking. Lipids were methylated with 2 mL 0.5 M sodium methoxide and 1 mL methanolic HCl (5% HCl vol/vol) at 50°C for 5 min, and FA composition was determined by GC (Ferlay et al., 2010). Apparent transfer rates (%) of LA (C18:2n-6) and ALA (C18:3n-3) were calculated as their excretion in milk, assuming 93.3% of milk fat is FA (Glasser et al., 2007), divided by FA intake. Data from one cow (treatment EL3, period 3) were excluded due to an aberrant milk FA profile.

Blood Plasma Metabolites. From all cows in both experiments, blood samples were collected from the coccygeal vessels on d 27 of each experimental period in 9-mL plasma tubes (Vacutainer K3EDTA, Vacuette, Greiner Bio-One GmbH). Samples were immediately placed on ice, centrifuged (3,500 × g, 15 min, 4°C), and plasma was stored at −20°C until analysis. Nonesterified fatty acids (NEFA), total cholesterol (TC), triacylglycerols (TAG), and acetate were analyzed by spectrophotometry on a clinical chemistry analyzer using commercial kits: NEFA (Sobioda, reagent 1: W1W434-91795; reagent 2: W1W436-91995), TC (Thermo-Fisher, reference 981813), TAG (Thermo-Fisher, reference 981786), and acetate (Thermo-Fisher, reference 984356), respectively.

Gas Measurements. An AHC unit (GreenFeed, C-Lock Inc., Rapid City, SD) was installed in the pen where all cows were housed throughout the experiments, except for 8 cows that were moved to tiestalls from d 21 to 27 of each period for nutrient digestibility and nitrogen partition measurements (Manzocchi et al., 2023). The AHC was always accessible except during milkings (0800 to 0830 and 1530 to 1600 h). Cows were accustomed to use the AHC during 3-wk adaptation period before the start of the experiments.

Spot samples of exhaled air were collected during the voluntary visits, limited to a maximum of 3 visits per cow per 24 h. During each visit, the AHC dispensed up to five 40-g drops of pelleted concentrate at 30-s intervals, with a maximum of 600 g/cow per day. The AHC unit performed daily autocalibration (0400 h) with a certified gas mixture containing CH₄ (504 ppm), CO₂ (5,020 ppm), H₂ (10.23 ppm) and O₂ (21.02 ppm) in nitrogen (Air Liquide, Mitry-Mory, France). Air filters were replaced and cleaned twice weekly or whenever air flow dropped below 27 L/s. Gas measurements were collected over 14 consecutive days in wk 3 and 4 of each experimental period, yielding 1,276 spot samples. Data from 3 rumen-cannulated cows included in Exp.1 were excluded to avoid biases caused by gas leakage through the cannula and associated distortion of eructation peak profiles (Martin et al., 2020b). Data from an additional cow included Exp.1 were excluded because of technical issues with animal identification. Outlier detection and

data processing followed Coppa et al. (2021), resulting in 1,058 valid spot samples: 283 in Exp.1 (n = 4 cows/treatment) and 775 in Exp.2 (n = 8 cows/treatment). The number of valid measurements averaged 18 ± 0.93 (mean $\pm$ SE) per cow and period in Exp.1 and 24 ± 1.83 per cow and period in Exp.2. Cows achieved, on average, 1.3 ± 0.05 visits/day in Exp.1 and 2.3 ± 0.04 visits/day in Exp.2, corresponding to 43% and 77% of the maximum visits offered per day, respectively. The interval between visits was on average 18 ± 25 h (mean $\pm$ SD) in Exp.1 and 13 ± 24 h in Exp.2. For each cow, mean values of daily CH₄ production (g CH₄/day) were calculated from all valid spot measurements in wk 3 and 4 of each period. Methane yield (g CH₄/kg DMI), CH₄ intensity (g CH₄/kg ECM), and GE losses as CH₄ (CH₄ energy/GE intake) were then calculated with DMI, ECM, and GE intake data averaged over wk 3 and 4, respectively.

Ruminal Digesta. Ruminal digesta were collected on d 25 and 27 of each experimental period from the 3 rumen-cannulated cows in Exp.1, immediately before the morning feeding (17 h after evening feeding) and 5 h after feeding. The rumen-cannulated cows were fed according to the experimental feeding schedule. Samples therefore represent ruminal FA profiles at 2 standardized postprandial times under practical feeding conditions. Approximately 500 g of digesta was collected from each rumen region (cranial, ventral, dorsal and caudal) by hand through the cannula, without draining liquid. All samples were pooled and carefully homogenized, and ~125 g were stored at −20°C for later lyophilization; the remaining material was returned to rumen. The FA profile of freeze-dried samples was analyzed by GC after lipid extraction and methylation, following Zened et al. (2011). Ruminal apparent changes in FA proportions were evaluated as the difference in relative abundance (as % of total FA) at 5 and 17 h after feeding.

Statistical Analysis

The experimental unit was the individual cow within each experiment. All analyses were conducted in R (version 4.3.2; R Core Team, 2022) using RStudio (RStudio Team, 2020). Linear mixed-effects models were fitted with the lmer function in the lme4 package (Bates et al., 2015), using restricted maximum likelihood and the lmerControl function with the “nloptwrap” optimizer. For milk FA profile and CH₄ production data, the model included the fixed effects of the dietary treatment (RL, EL1, EL2, and EL3 in Exp.1; RLS, ELS1, ELS2, and ELS3 in Exp.2), and square (1, 2), with cow (1–8) nested within square and experimental period (1–4) as random effects. For ruminal FA profile, linear mixed-effects models were fitted using restricted maximum likelihood in the nlme package (Pinheiro et al., 2025) to evaluate

Table 1. Chemical composition, fatty acid profile, and particle size distribution of the raw or extruded white lupines without (Exp.1) or with (Exp.2) the addition of reducing sugars, adapted from Manzocchi et al. (2023)

Item	Lupines (Exp.1) ¹				Lupines + reducing sugars (Exp.2) ²			
	RL	EL1	EL2	EL3	RLS	ELS1	ELS2	ELS3
Chemical composition, g/kg DM								
OM	866	898	903	922	863	897	901	920
CP	345	354	355	344	336	349	353	344
NDF	235	228	227	221	235	213	211	206
Ether extract ³	94	95	97	109	92	94	95	126
Total fatty acids, g/kg DM	81.9	69.7	71.0	81.6	77.9	74.7	95.4	98.1
C16:0	7.1	5.8	5.9	7.6	6.9	6.3	7.8	8.0
C18:0	1.6	1.4	1.4	2.4	1.5	1.5	1.9	2.3
<i>cis</i> -9 C18:1	39.3	32.7	32.7	33.3	37.6	37.6	43.6	39.5
C18:2n-6 (LA)	14.9	12.8	12.7	12.7	14.2	13.6	17.4	17.8
C18:3n-3 (ALA)	11.5	11.1	11.8	19.7	10.7	11.4	16.1	23.2
Other FA	7.7	5.8	6.3	5.9	7.1	4.5	8.6	7.4
Particle size distribution, %								
>1 mm/<1 mm	48/52	53/47	64/36	38/62	48/52	50/50	41/59	46/54

¹RL: raw lupine seeds; EL1: lupine seeds extruded at 115°C; EL2: lupine seeds extruded at 135°C; EL3: lupine seeds extruded at 150°C.

²RLS: raw lupine seeds with addition of reducing sugars; ELS1: lupine seeds added with reducing sugars extruded at 115°C; ELS2: lupine seeds added with reducing sugars extruded at 135°C; ELS3: lupine seeds added with reducing sugars extruded at 150°C.

³After acid hydrolysis.

the effect of dietary treatment, period, sampling time, including the treatment $\times$ time interaction. Period and cow were specified as a random effect to account for repeated measurements within animals. Within each cow and period, the repeated observations were averaged by sampling time. Estimated marginal means (least squares means) and Tukey-adjusted pairwise comparisons were obtained with the emmeans package (Lenth, 2023). Significance was declared at $P < 0.05$ and tendencies at $0.05 \leq P < 0.10$. Degrees of freedom were estimated with the Kenward–Roger method. Normality of residuals was assessed with the Shapiro–Wilk test, and homoscedasticity with the ncvTest function. Estimated marginal means and SEM are reported in tables, and Tukey-adjusted P -values for multiple comparisons are presented in the Results.

A post hoc power analysis was performed to assess the ability to detect treatment effects on FA proportions in ruminal digesta. Calculations were based on the observed SEM for C18:0 (1.25 g/100 g FA), *trans*-11 C18:1 (0.42 g/100 g FA), C18:2n-6 (0.63 g/100 g FA), and C18:3n-3 (0.19 g/100 g FA), the number of cows per treatment ($n = 3$), and 2 sampling times per period. Analyses were conducted in R (version 4.3.3) using the pwr package (Champany et al., 2025), assuming $\alpha = 0.05$ for 2-sided tests.

RESULTS

Lupine Fatty Acid Profile, Particle Size Distribution, and Experimental Diets

Chemical composition and FA concentrations in the raw and extruded lupines are reported in Table 1. On average, raw and extruded whole white lupine seeds

contained 99 g EE/kg DM and 102 g EE/kg DM in Exp.1 and Exp.2, respectively. The main FA in lupines were *cis*-9 C18:1 (45.2% of total FA), C18:3n-3 (18.3% of total FA), C18:2n-6 (17.7% of total FA), and C16:0 (8.6% of total FA). Lupines extruded at 150°C with or without adding reducing sugars had a numerically higher proportion of C18:3n-3 (on average, +6.8 g/100 g of FA in Exp.1, +7.2 g/100 g of FA in Exp.2) than all other lupines. Chemical composition and FA profile and concentration in the rations are reported in Table 2. In Exp.1, lupines contributed to 47%, 47%, 48%, and 51% of dietary EE in the RL, EL1, EL2, and EL3 diets, respectively. In Exp.2, the contributions were 46%, 47%, 47%, and 54% of dietary EE for the RLS, ELS1, ELS2, and ELS3 diets, respectively. For *cis*-9 C18:1, lupines supplied 82%, 79%, 79%, and 79% of dietary FA intake in the RL, EL1, EL2, and EL3 diets of Exp.1, and 81%, 81%, 83%, and 82% in the RLS, ELS1, ELS2, and ELS3 diets of Exp.2. Regarding C18:3n-3, lupines provided 50%, 49%, 50%, and 63% of dietary FA intake in the RL, EL1, EL2, and EL3 diets of Exp.1, and 58%, 48%, 49%, and 58% in the RLS, ELS1, ELS2, and ELS3 diets of Exp.2. For C18:2n-6, the corresponding contributions to dietary FA intake were 44%, 40%, 40%, and 40% in Exp.1, and 43%, 42%, 48%, and 48% in Exp.2.

The particle size distribution of the raw and extruded lupines was affected by the extrusion temperature and, to a lesser extent, by the addition of reducing sugars (Table 1). Raw lupines (RL and RLS) showed relatively coarse and heterogeneous particle sizes, with a numerically higher proportion of larger particles (48% >1 mm) than extruded lupines. Extrusion progressively reduced particle size as temperature increased, as shown by

Table 2. Chemical composition of experimental diets¹ containing raw or extruded white lupines without (Exp.1) or with addition of reducing sugars (Exp.2), adapted from Manzocchi et al. (2023)

Item	Lupines (Exp.1) ²				Lupines + reducing sugars (Exp.2) ³			
	RL	EL1	EL2	EL3	RLS	ELS1	ELS2	ELS3
Chemical composition, g/kg DM								
OM	924	924	924	923	924	924	924	923
CP	154	154	156	154	152	155	156	154
NDF	480	476	476	481	477	477	476	479
Ether extract ⁴	40	40	40	43	40	40	40	46
Gross energy, MJ/kg DM	18.6	18.8	18.8	18.8	18.9	18.8	18.8	18.9
Total fatty acids, g/kg DM	35.3	32.9	33.1	35.3	34.5	33.9	38.0	38.5
C16:0	5.1	4.8	4.8	5.2	5.0	4.9	5.2	5.3
C18:0	0.70	0.68	0.68	0.87	0.70	0.68	0.78	0.85
<i>cis</i> -9 C18:1	10.5	9.2	9.2	9.3	10.2	10.2	11.4	10.6
C18:2n-6 (LA)	8.5	8.1	8.1	8.1	8.4	8.2	9.0	9.1
C18:3n-3 (ALA)	6.2	6.1	6.3	7.9	6.1	6.2	7.1	8.6
Nutritive value ⁵								
ME, MJ/kg DM	10.7	10.7	10.7	10.6	10.7	10.7	10.6	10.6
NEL, ⁶ UFL/kg DM	0.92	0.92	0.92	0.91	0.92	0.92	0.92	0.92
MP, ⁷ g PDI/kg DM	80	91	92	93	79	90	91	93
RPB, ⁸ g/kg DM	24	16	13	7	20	14	11	9

¹Ingredients of the experimental diets on a DM basis: 30% corn silage (per kg DM: 86 g CP, 345 g starch, 387 g NDF, 49 g ash), 24% grass silage (per kg DM: 127 g CP, 552 g NDF, 118 g ash), 15% hay (per kg DM: 70 g F, 586 g NDF, 75 g ash), 11% concentrate (per kg DM: 177 g CP, 355 g starch, 206 g NDF, 79 g ash), and 20% experimental lupines. Concentrate consisted on a DM basis of 30% sugar beet pulp, 22.7% wheat, 20% barley, 15% rapeseed meal, 7.9% soybean meal, 1.5% sugar molasses, 1.0% dicalcium phosphate, 1.0% magnesia, 0.9% NaCl, 1,000 IU/kg vitamin A, 1,400 IU/kg vitamin D₃, 15 IU/kg vitamin E, 0.75 mg/kg I, 0.26 mg/kg Co, 15 mg/kg Cu, 60 mg/kg Mn, 90 mg/kg Zn, and 0.25 mg/kg Se. In addition, 200 g/d of mineral-vitamin mix (20% Ca, 2.5% P, 4.5% Mg, 3.5% Na, 1% S, 400,000 IU/kg of vitamin A, 120,000 IU/kg of vitamin D₃, 1,600 IU/kg of vitamin E, 1.3 g/kg of Cu, 5 g/kg of Zn, 3.5 g/kg of Mn, 90 mg/kg of I, 36 mg/kg of Co, and 20 mg/kg of Se; Galaphos MiDi Duo, CCPA) was fed.

²RL: raw lupines; EL1: lupines extruded at 115°C; EL2: lupines extruded at 135°C; EL3: lupines extruded at 150°C.

³RLS: raw lupines with addition of reducing sugars; ELS1: lupines added with reducing sugars extruded at 115°C; ELS2: lupines added with reducing sugars extruded at 135°C; ELS3: lupines added with reducing sugars extruded at 150°C.

⁴After acid hydrolysis.

⁵Estimated according to INRA (2018) based on a feeding level of DM corresponding to 3.1% BW/d (DMI = 22.0 kg/d, BW = 700 kg) and actual feed chemical composition.

⁶Net energy for lactation expressed in Unité Fourragère Lait; 1 UFL = 7.37 MJ.

⁷MP expressed in grams of protein truly digestible in the intestine (PDI) according to INRA (2018).

⁸Rumen protein balance.

numerically increasing proportions of particles <1 mm with lupines extruded at 150°C without the addition of reducing sugars, as compared with raw lupines. The lupines extruded at 150°C exhibited the finest and most fragmented material also within particles smaller than 1 mm (Supplemental Table S1, see Notes).

Ruminal Biohydrogenation of Polyunsaturated Fatty Acids

The C18 FA profile in ruminal digesta of the 3 cannulated cows in Exp.1, sampled during wk 4 at 5 h and 17 h after feeding, is reported in Table 3, with additional FA data in the supplemental material (Supplemental Table S3, see Notes). Apparent temporal changes of RBH precursors, intermediates, and final products are shown in Figure 1. At 5 h after feeding, *cis*-9 C18:1 was lower with EL1 ($P = 0.03$) and EL2 ($P = 0.01$) than with RL, but proportions converged across treatments by 17 h after feeding. *Trans*-11 C18:1 was consistently higher ($P <$

0.01) in ruminal digesta of cows fed EL3 than in all other treatments, irrespective of sampling time. The proportion of LA did not differ among treatments ($P = 0.63$) but decreased ($P < 0.01$) by an average of 52% from 5 to 17 h after feeding. Proportions of *cis*-9,*trans*-11 C18:2 and *trans*-11,*cis*-15 C18:2 were higher at 5 h with EL3 (both $P < 0.01$) but decreased with time. The proportion of ALA decreased from 5 to 17 h with RL ($P = 0.01$), EL1 ($P < 0.01$), and EL3 ($P < 0.01$), whereas the decrease with EL2 ($P = 0.06$) was only numerical. Disappearance of *trans*-11 C18:1 was significantly different ($P = 0.01$) with all extruded lupines compared with RL (Table 4), whereby *trans*-11 C18:1 proportion in ruminal digesta increased from 5 to 17 h after feeding with RL (Table 3). The disappearance of *cis*-9,*trans*-11 C18:2 was higher ($P = 0.04$) with EL3 than with RL. Other precursors and intermediates of RBH did not differ among treatments. The proportions of the final product C18:0 were unaffected by diet ($P = 0.36$; Table 4) but increased across all treatments from 5 to 17 h after feeding ($P < 0.01$; Table 3).

Milk Fatty Acid Profile

The effects of dietary treatments on the sums of FA in milk fat were largely consistent across both experiments (Table 5). Feeding lupines extruded at 150°C, with (ELS3) or without (EL3) added sugars, significantly decreased the proportion of SFA in milk fat compared with all other dietary treatments (−4.7 g/100 g of total FA in Exp.1 and −3.4 g/100 g of total FA in Exp.2; both $P < 0.001$). Even-chain FA (C6:0 to C16:0) tended to be lower with EL3 than RL in Exp.1 ($P = 0.06$, −3.4 g/100 g of total FA) and were significantly lower with ELS3 than with RLS, ELS1, and ELS2 in Exp.2 ($P < 0.001$; −3.7 g/100 g of total FA). This effect was mainly driven by C16:0, which was reduced in milk with EL3 (−2.3 g/100 g of total FA; $P < 0.01$) and with ELS3 (−2.9 g/100 g of total FA; $P < 0.01$) compared with all other diets (Table 6). In contrast, odd-chain FA ($P = 0.13$ in Exp.1, $P = 0.91$ in Exp.2), *iso* branched-chain FA ($P = 0.13$ in Exp.1, $P = 0.14$ in Exp.2), and *anteiso* branched-chain FA ($P = 0.95$ in Exp.1, $P = 0.39$ in Exp.2) did not differ among dietary treatments in either experiment. By contrast, *trans* MUFA proportions were markedly higher in the milk fat of cows fed EL3 and ELS3 compared with those fed lupines extruded at 115°C or 135°C (all $P < 0.01$; Table 5). In Exp.1, EL3 increased *trans*-10 C18:1, *trans*-11 C18:1, *trans*-12 C18:1, and *trans*-16 C18:1 (all $P < 0.05$) relative to RL, with intermediate values for EL1 and EL2. In Exp.2, ELS3 increased *trans*-10 C18:1, *trans*-12 C18:1, and *trans*-16 C18:1 (all $P < 0.05$), whereas *trans*-11 C18:1 was not affected ($P = 0.14$; Table 6). Minor *cis* MUFA isomers (i.e., *cis*-11, *cis*-12, *cis*-13, *cis*-15, and *cis*-16 C18:1) were higher with EL3 than with RL in Exp.1 (all $P < 0.05$; Table 6), but total *cis* MUFA, including the main isomer *cis*-9 C18:1 (21.1 g/100 g of FA), did not differ among treatments ($P = 0.92$; Table 5). Similarly, in Exp.2, *cis*-12 C18:1, *cis*-15 C18:1, and *cis*-16 C18:1 were higher (all $P < 0.01$) with ELS3 compared with RLS, with intermediate values for ELS1 and ELS2. *Cis*-9 C18:1 tended ($P = 0.05$) to be higher with ELS3 than with RLS (Table 6).

The proportions of PUFA in milk fat were higher ($P < 0.01$) in cows fed EL3 (+0.9 g/100 g FA) or ELS3 (+0.7 g/100 g FA) compared with all other treatments (Table 5). This increase was reflected in both n-3 FA (+0.32 g/100 g FA, +0.21 g/100 g FA in Exp.1 and Exp.2, respectively) and n-6 FA (+0.18 g/100 g FA in both experiments; all $P < 0.01$) with EL3 and ELS3 compared with all other treatments in the respective experiment. Similarly, the sum of CLA isomers was highest with EL3 and ELS3 ($P < 0.01$), primarily due to *cis*-9,*trans*-11 C18:2, which increased by +0.38 g/100 g FA in Exp.1 and +0.29 g/100 g FA in Exp.2 relative to raw lupines.

The ratio of *cis*-9 C14:1 to *cis*-9 C14:1+C14:0 did not differ among dietary treatments in either experiment ($P = 0.42$ in Exp.1 and $P = 0.28$ in Exp.2; Table 5). Daily LA yield was unaffected in Exp.1 ($P = 0.46$) but was higher with ELS3 (+1.5 g/d; $P < 0.01$) than with ELS1 and ELS2 in Exp.2 (Table 5). Correspondingly, the apparent LA transfer rate averaged 5.2% in Exp.1 and significantly differed among treatments ($P = 0.03$). In Exp.2, the LA transfer rate was 0.7 percentage points greater ($P < 0.01$) with RLS and ELS3 than with ELS1 and ELS2.

Daily ALA yield was increased with EL3 (+2.1 g/d; $P < 0.01$) in Exp.1 and with ELS3 (+1.5 g/d; $P < 0.001$) in Exp.2 compared with all other treatments. Accordingly, the apparent ALA transfer rate was 0.8 percentage points higher ($P < 0.01$) with the EL3 than with all other dietary treatments in Exp.1. In Exp.2, the apparent ALA transfer rate averaged 3.2% and was higher ($P = 0.03$) with RL than with ELS2. Further treatment means and effects on additional individual, minor FA not reported in Table 6 are provided in Supplemental Table S2 (see Notes).

Blood Metabolites and CH₄ Production

Plasma concentrations of NEFA, TAG, TC, and acetate before morning feeding did not differ among dietary treatments in either experiment (all $P > 0.05$; Table 7). Methane production did not significantly differ among dietary treatments in either experiment ($P = 0.28$ in Exp.1, $P = 0.70$ in Exp.2; Table 7), averaging 437 ± 23.3 g/d (CV = 26.4%) in Exp.1 and 477 ± 23.4 g/d (CV = 23.9%) in Exp.2. Similarly, CH₄ yield (g/kg DMI) was unaffected ($P = 0.54$ in Exp.1, $P = 0.62$ in Exp.2). However, CH₄ intensity was lower with EL3 (−1.8 g CH₄/kg ECM) than with all other treatments in Exp.1 ($P < 0.01$), but did not differ in Exp.2 ($P = 0.24$). Gross energy losses as CH₄ averaged 5.7% of GE intake in Exp.1 and 6.7% in Exp.2, with no dietary effect.

DISCUSSION

The primary aim of the present study was to investigate the effects of extrusion and extrusion temperature of whole white lupines, with or without addition of reducing sugars, on (1) the RBH of PUFA, (2) the milk FA profile, and (3) the transfer rate of PUFA to milk. Although not a central focus of the study, CH₄ emissions were also measured, given that extrusion of certain oilseeds has been reported to exert mitigating effects on ruminal CH₄ production (Martin et al., 2008, 2020a). Moreover, a previous study (Manzocchi et al., 2023) presented complementary measurements on the effects of extrusion and extrusion temperature of white lupines on MP supply, nutrient digestibility, N use efficiency, and N

Table 3. C18 fatty acids (g/100 g total FA) in ruminal digesta sampled at 5 h and 17 h after feeding in 3 rumen-cannulated cows fed raw or extruded white lupines without reducing sugars (Exp.1); effects of dietary treatment, sampling time, and their interaction are shown

Fatty acids (g/100 g of FA)	Lupines ¹ (Exp.1)										P-value ²				
	RL			EL1		EL2		EL3							
	5 h	17 h		5 h	17 h		5 h	17 h		5 h	17 h	SEM	Trt	Time	Trt × Time
C18:0	40.7	50.4		41.2	51.2		43.1	49.8		38.3	49.8	1.25	0.10	<0.01	0.13
<i>cis</i> -9 C18:1	9.98 ^a	3.31 ^c		7.54 ^b	2.96 ^c		6.64 ^b	3.64 ^c		8.07 ^{ab}	3.22 ^c	0.473	<0.01	<0.01	<0.01
<i>cis</i> -11 C18:1	0.74 ^a	0.40 ^c		0.64 ^{ab}	0.38 ^c		0.60 ^b	0.64 ^c		0.68 ^{ab}	0.42 ^c	0.037	0.04	<0.01	<0.01
<i>cis</i> -12 C18:1	0.35	0.24		0.46	0.25		0.43	0.27		0.59	0.33	0.033	<0.01	<0.01	0.17
<i>trans</i> -6/-7/-8 C18:1	0.47 ^{cd}	0.43 ^d		0.83 ^a	0.58 ^{bcd}		0.77 ^{ab}	0.57 ^{bcd}		0.89 ^a	0.71 ^{abc}	0.063	<0.01	<0.01	0.23
<i>trans</i> -9 C18:1	0.29	0.25		0.49	0.34		0.46	0.34		0.52	0.45	0.040	<0.01	<0.01	0.33
<i>trans</i> -10 C18:1	0.47 ^d	0.43 ^d		0.83 ^{ab}	0.58 ^{cd}		0.73 ^{ab}	0.55 ^{cd}		0.86 ^a	0.68 ^{bc}	0.065	<0.01	<0.01	0.03
<i>trans</i> -11 C18:1	2.12	2.49		3.24	3.22		3.16	3.17		4.78	4.72	0.416	<0.01	0.73	0.88
<i>trans</i> -12 C18:1	0.58	0.50		0.86	0.58		0.79	0.58		1.01	0.77	0.060	<0.01	<0.01	0.22
<i>trans</i> -13/14 C18:1	1.11 ^{cd}	1.01 ^d		1.69 ^{abc}	1.24 ^{bcd}		1.57 ^{abcd}	1.19 ^{bcd}		2.21 ^a	1.85 ^{ab}	0.155	<0.01	<0.01	0.54
<i>trans</i> -15 C18:1	0.47	0.46		0.64	0.52		0.64	0.52		0.80	0.77	0.058	<0.01	0.07	0.64
<i>trans</i> -16 C18:1	0.51	0.54		0.62	0.61		0.62	0.57		0.76	0.84	0.052	<0.01	0.67	0.45
<i>cis</i> -9, <i>cis</i> -12 C18:2 (LA)	5.45	2.59		5.13	2.25		4.99	2.59		5.61	2.30	0.368	0.63	<0.01	0.73
<i>trans</i> -11, <i>cis</i> -15 C18:2	0.32 ^{bc}	0.29 ^c		0.48 ^b	0.34 ^{bc}		0.41 ^{bc}	0.29 ^c		0.89 ^a	0.46 ^b	0.046	<0.01	<0.01	<0.01
<i>trans</i> -10, <i>cis</i> -12 C18:2	0.04 ^{bc}	0.04 ^{bc}		0.07 ^a	0.03 ^c		0.06 ^{ab}	0.03 ^c		0.07 ^{abc}	0.03 ^c	0.005	0.04	<0.01	<0.01
<i>cis</i> -9, <i>trans</i> -11 C18:2	0.06 ^b	0.07 ^b		0.12 ^{ab}	0.09 ^b		0.11 ^{ab}	0.10 ^{ab}		0.23 ^a	0.11 ^{ab}	0.026	<0.01	0.04	0.07
<i>trans</i> 9, <i>trans</i> 11 C18:2	0.04	0.03		0.04	0.02		0.05	0.03		0.05	0.04	0.007	<0.01	<0.01	0.62
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3 (ALA)	3.26 ^b	1.82 ^c		3.65 ^{ab}	1.68 ^c		2.98 ^b	1.80 ^c		4.45 ^a	1.75 ^c	0.191	0.02	<0.01	<0.01

^{a-d}Values with differing superscripts significantly differ ($P < 0.05$) from each other due to treatment × time after feeding interaction according to Tukey-corrected multiple comparisons among least squares means.

¹RL: raw lupines; EL1: lupines extruded at 115°C; EL2: lupines extruded at 135°C; EL3: lupines extruded at 150°C.

²Probability of effects: Trt = dietary treatment (RL, EL1, EL2, EL3); Time = sampling time after feeding (5 h or 17 h); Trt × Time = interaction between dietary treatment and sampling time.

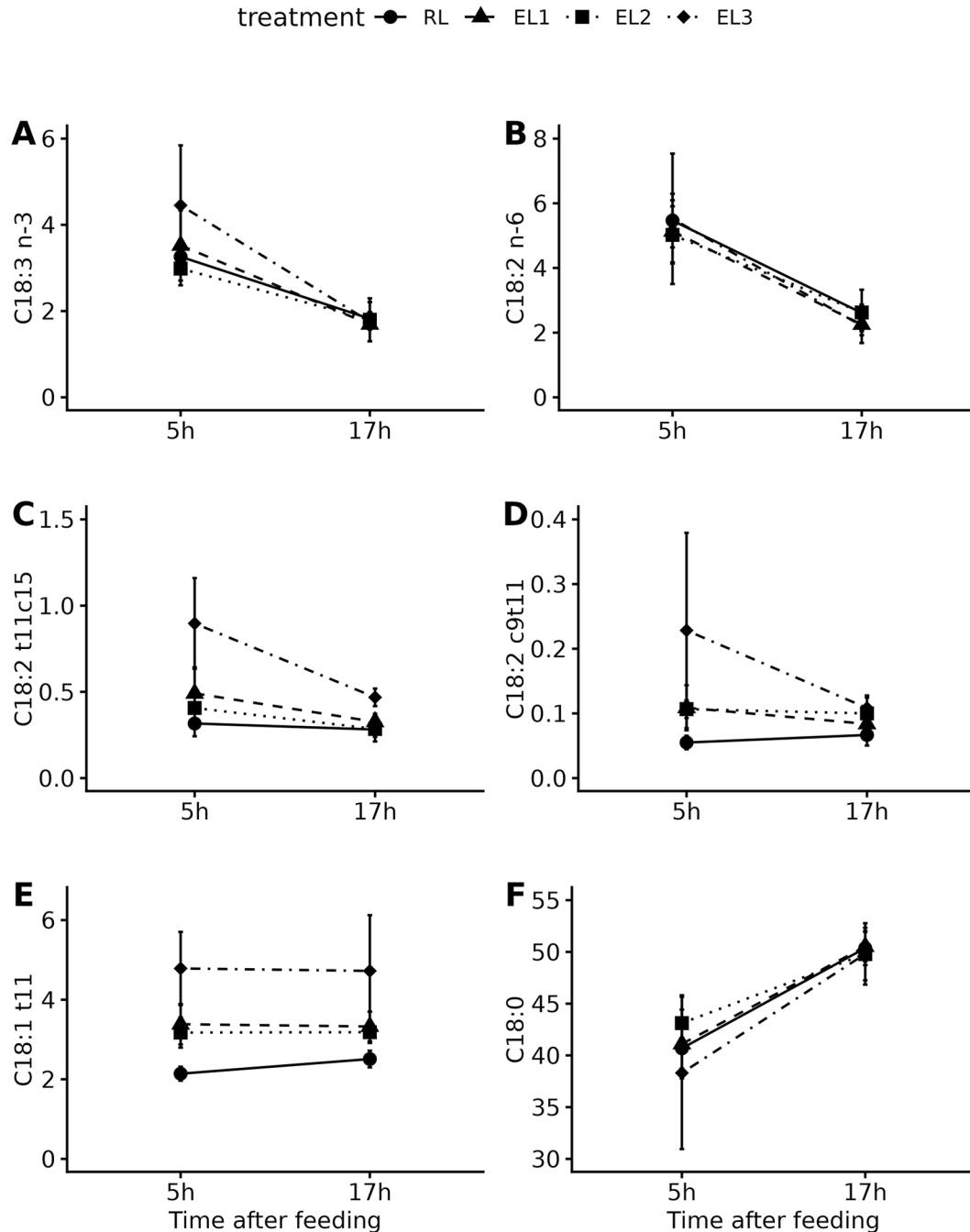


Figure 1. Mean concentration (in g/100 g of total fatty acids) and SE of biohydrogenation precursors, intermediates, and final product in ruminal digesta, sampled twice during wk 4 of each experimental period on 3 rumen-cannulated cows fed raw or extruded lupines without addition of reducing sugars, 5 h and 17 h after feeding. (A) C18:3n-3, (B) C18:2n-6, (C) *trans*-11,*cis*-15 C18:3 (C18:2t11c15), (D) *cis*-9,*trans*-11 C18:2 (C18:2c9t11), (E) *trans*-11 C18:1 (C18:1t11); (F): C18:0. RL = raw white lupine seeds; EL1 = lupines extruded at 115°C; EL2 = lupines extruded at 135°C; EL3 = lupines extruded at 150°C.

and carboxymethyl-lysine partitioning and excretion, as well as ruminal fermentation (ruminal VFA profile, and ammonia concentration) and kinetics of ruminal parameters on rumen-cannulated cows conducted in the frame of these experiments.

Ether Extract and Fatty Acid Content of Extruded Lupines

Lupines extruded at 150°C showed numerically higher proportions of EE and ALA. This difference likely re-

Table 4. Apparent relative temporal changes in proportions of precursors, intermediates, and final products of ruminal biohydrogenation (% of FA disappeared or appeared over 12 h) measured during wk 4 of each period in 3 rumen-cannulated cows fed raw or extruded white lupines without reducing sugars included (Exp.1); dietary treatment means are shown

Item	Lupines ¹ (Exp.1)				SEM	P-value
	RL	EL1	EL2	EL3		
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3 (ALA)	-46	-50	-39	-54	7.0	0.44
<i>cis</i> -9, <i>cis</i> -12 C18:2 (LA)	-47	-54	-53	-52	6.9	0.83
<i>cis</i> -9, <i>trans</i> -11 C18:2 (CLA)	22 ^a	-13 ^{ab}	-9.3 ^{ab}	-38 ^b	13.6	0.04
<i>trans</i> -11, <i>cis</i> -15 C18:2	-11	-30	-27	-44	7.8	0.05
<i>trans</i> -11 C18:1	15 ^a	-3.7 ^b	-1.0 ^b	-0.6 ^b	4.5	0.01
<i>cis</i> -9 C18:1	-67	-60	-45	-53	6.2	0.14
C18:0	25	24	15	35	7.8	0.36

^{a,b}Values with differing superscripts significantly differ ($P < 0.05$) from each other according to Tukey-corrected multiple comparisons.

¹RL: raw lupines; EL1: lupines extruded at 115°C; EL2: lupines extruded at 135°C; EL3: lupines extruded at 150°C.

flects analytical extractability rather than a true biological increase in lipid content. Higher extrusion temperatures can modify lipid-protein interactions (Hülsebusch et al., 2025) and increase the mechanical disruption of the seed matrix, thereby increasing lipid recovery during acid hydrolysis. In the present study, extrusion at 150°C produced the smallest particle size (lowest diameter at 50% of the cumulative volume distribution [Dv50] and highest proportions of particles <1 mm), indicating a much finer and more fragmented material compared with lupines extruded at 115°C or 135°C. Such reductions in particle size are consistent with previous observations in other oleo-proteaginous seeds (Chapoutot et al., 2018) and may directly facilitate more efficient solvent penetration and lipid liberation during extraction. Although a biological contribution cannot be entirely excluded (e.g., through lipase inactivation or oleosome disruption), the available data suggest that the numerically higher EE and ALA at 150°C most likely reflect greater extractability rather than an actual increase in true lipid content.

Effect of Lupine Extrusion on Ruminal Biohydrogenation of Polyunsaturated Fatty Acids

In the cannulated cows included in Exp.1, extrusion conditions of white lupines modulated the postprandial proportions of ruminal C18 FA without altering the final product of RBH (C18:0). At 5 h after feeding, *cis*-9 C18:1 (oleic acid) was lower with EL1 and EL2 than with RL. However, RL also provided a greater initial concentration of *cis*-9 C18:1 in the diet, and therefore the higher ruminal proportion observed 5 h after feeding may partly reflect differences in dietary supply rather than differences in the rate of RBH. By 17 h after feeding, treatment differences disappeared, consistent with convergence of the *cis*-9 C18:1 pool over the diurnal cycle.

In contrast, *trans*-11 C18:1 (vaccenic acid) was consistently higher with EL3 across both sampling times, accompanied at 5 h by higher *cis*-9,*trans*-11 C18:2 (CLA) and *trans*-11,*cis*-15 C18:2, indicating that extrusion at 150°C (EL3) promoted accumulation of RBH intermediates rather than complete biohydrogenation to C18:0. The apparent decline of the LA proportion (on average 52% from 5 h to 17 h across all treatments) and the decrease of ALA across most diets reflect ongoing biohydrogenation. Although the disappearance of *trans*-11 C18:1 differed for all extruded diets versus RL, and RL showed an increase in *trans*-11 C18:1 from 5 h to 17 h, the C18:0 proportions were unaffected by diet and increased with time in all treatments, implying that extrusion primarily shifts the timing and distribution of intermediates rather than the overall extent of hydrogenation within the 12-h window between the 2 sampling time points. Collectively, these patterns indicate that extrusion at 150°C without reducing sugars (EL3) channeled the flux through the vaccenic to CLA pathway, which is consistent with the observed higher proportions of CLA precursors in rumen digesta 5 h after feeding and is predictive of higher milk *cis*-9,*trans*-11 CLA via mammary $\Delta 9$ -desaturation of vaccenic acid (Griinari et al., 2000).

In addition to chemical changes induced by extrusion, differences in particle size among treatments may also have contributed to the observed patterns of ruminal FA metabolism. Particle size can influence the accessibility of lipids to rumen microbes, the specific gravity of feed particles, and their retention time within the rumen. Finer particles generally increase the surface area available for microbial colonization and may accelerate lipid release from the feed matrix, thereby increasing the exposure of UFA to ruminal biohydrogenation. Conversely, larger particles may remain associated with the solid digesta

Table 5. Sums of fatty acids (g/100 g of fatty acids) in milk fat and apparent transfer rates of linoleic (LA) and α -linolenic acids (ALA) in dairy cows fed raw or extruded white lupines without (Exp.1; n = 8) or with the addition of reducing sugars (Exp.2; n = 8)

Item ³	Lupines (Exp.1) ¹				SEM	P-value	Lupines + Reducing sugars (Exp.2) ²					SEM	P-value
	RL	EL1	EL2	EL3			RLS	ELS1	ELS2	ELS3			
SFA	67.9 ^a	65.6 ^a	66.7 ^a	62.0 ^b	1.05	<0.01	68.7 ^a	66.6 ^a	67.3 ^a	64.1 ^b	0.91	<0.01	
Even-chain (C6:0-C16:0) FA	50.1	47.6	48.7	45.4	1.72	0.06	51.0 ^a	48.9 ^a	49.8 ^a	46.2 ^b	0.90	<0.01	
Odd-chain FA	1.90	1.80	1.88	1.74	0.081	0.13	1.74	1.78	1.74	1.73	0.080	0.91	
Iso branched FA	1.15	1.19	1.17	1.02	0.076	0.13	1.05	1.15	1.07	1.00	0.050	0.14	
Anteiso branched FA	1.41	1.51	1.38	1.49	0.255	0.95	1.41	1.55	1.45	1.70	0.142	0.39	
cis MUFA	24.5	25.9	25.1	25.2	1.79	0.92	23.8	25.0	24.1	25.8	0.765	0.09	
trans MUFA	1.52 ^c	2.17 ^b	2.04 ^b	2.96 ^a	0.136	<0.01	1.37 ^c	1.99 ^b	2.10 ^b	2.58 ^a	0.094	<0.01	
PUFA	2.44 ^b	2.63 ^b	2.59 ^b	3.49 ^a	0.217	<0.01	2.45 ^b	2.58 ^b	2.74 ^b	3.32 ^a	0.093	<0.01	
n-3	0.73 ^b	0.74 ^b	0.73 ^b	1.05 ^a	0.043	<0.01	0.70 ^b	0.72 ^b	0.77 ^b	0.94 ^a	0.023	<0.01	
n-6	1.30 ^b	1.26 ^b	1.25 ^b	1.45 ^a	0.061	<0.01	1.33 ^b	1.28 ^b	1.33 ^b	1.49 ^a	0.030	<0.01	
CLA ⁴	0.32 ^c	0.49 ^b	0.46 ^b	0.72 ^a	0.034	<0.01	0.31 ^c	0.42 ^b	0.45 ^b	0.60 ^a	0.039	<0.01	
NI	0.37	0.35	0.32	0.45	0.045	0.15	0.47	0.34	0.40	0.43	0.063	0.22	
cis-9 C14:1 / (cis-9 C14:1 + C14:0)	0.082	0.084	0.083	0.079	0.0061	0.42	0.073	0.076	0.074	0.076	0.0051	0.28	
n-6/n-3 ratio	1.78 ^a	1.69 ^a	1.72 ^a	1.38 ^b	0.058	<0.01	1.91 ^a	1.79 ^a	1.75 ^a	1.59 ^b	0.043	<0.01	
FA yield, g/d	10.1	9.2	9.5	10.2	0.50	0.46	9.5 ^{ab}	8.5 ^b	8.9 ^b	10.2 ^a	0.46	<0.01	
C18:2n-6	4.7 ^b	4.9 ^b	4.6 ^b	6.8 ^a	0.35	<0.01	4.3 ^b	4.1 ^b	4.6 ^b	5.8 ^a	0.29	<0.01	
C18:3n-3													
Transfer rates (%)													
C18:2n-6 (LA)	5.0 ^b	5.1 ^{ab}	5.0 ^b	5.8 ^a	0.33	0.03	5.4 ^a	5.0 ^{ab}	4.7 ^b	5.3 ^a	0.20	<0.01	
C18:3n-3 (ALA)	3.3 ^b	3.4 ^b	3.2 ^b	4.1 ^a	0.18	<0.01	3.4 ^a	3.2 ^{ab}	3.0 ^b	3.2 ^{ab}	0.19	0.03	

^{a-c}Values with differing superscripts significantly differ ($P < 0.05$) from each other according to Tukey-corrected multiple comparisons.¹RL: raw lupines, EL1: lupines extruded at 115°C, EL2: lupines extruded at 135°C, EL3: lupines extruded at 150°C.²RLS: raw lupines with addition of reducing sugars; ELS1: lupines added with reducing sugars extruded at 115°C, ELS2: lupines added with reducing sugars extruded at 135°C, ELS3: lupines added with reducing sugars extruded at 150°C.³FA: fatty acids; NI: not identified.⁴Sum of *cis-9,trans-11* C18:2 (+*trans-7,cis-9+trans-8,cis-10*) + *trans-11,cis-13* C18:2 (+*cis-9,cis-11* + C21:0) + *trans-11,trans-13* C18:2 + *trans-9,trans-11* C18:2 (+*trans10,trans12+trans8,trans10*).

Table 6. Selected milk fatty acids (g/100 g of total fatty acids [FA]) in lactating cows fed raw or extruded white lupines without (Exp.1; n = 8) or with the addition of reducing sugars (Exp.2; n = 8)

Fatty acids (g/100 g of FA)	Lupines (Exp. 1) ¹				SEM	P-value	Lupines + reducing sugars (Exp.2) ²				SEM	P-value
	RL	EL1	EL2	EL3			RLS	ELS1	ELS2	ELS3		
C14:0	11.1	10.8	11.0	10.4	0.474	0.60	11.1	11.0	11.5	10.8	0.238	0.06
<i>cis</i> -9 C14:1	0.98	0.99	0.99	0.92	0.098	0.57	0.87	0.91	0.92	0.90	0.072	0.46
C15:0 <i>iso</i>	0.28	0.26	0.26	0.24	0.015	0.06	0.28 ^a	0.26 ^a	0.26 ^{ab}	0.24 ^b	0.006	<0.01
C15:0	1.10	1.03	1.10	1.00	0.058	0.16	1.04	1.01	1.06	1.00	0.038	0.11
C16:0	29.2 ^a	27.5 ^{ab}	28.0 ^{ab}	25.9 ^b	1.20	0.01	29.9 ^a	28.2 ^a	28.2 ^a	25.9 ^b	0.894	<0.01
C18:0	12.0	12.3	12.2	13.2	0.606	0.07	11.9	12.0	11.9	12.2	0.606	0.66
<i>cis</i> -9 C18:1 ³	20.5	21.9	21.0	21.0	1.75	0.92	20.3	21.2	20.3	22.0	0.653	0.05
<i>cis</i> -11 C18:1	0.62 ^b	0.69 ^{ab}	0.65 ^{ab}	0.80 ^a	0.060	0.04	0.63	0.68	0.67	0.72	0.035	0.20
<i>cis</i> -12 C18:1	0.19 ^c	0.24 ^b	0.23 ^{ab}	0.27 ^a	0.014	<0.01	0.19 ^c	0.23 ^b	0.24 ^{ab}	0.28 ^a	0.012	<0.01
<i>cis</i> -13 C18:1	0.06 ^b	0.08 ^{ab}	0.07 ^{ab}	0.09 ^a	0.010	0.02	0.06	0.08	0.08	0.09	0.009	0.06
<i>cis</i> -15 C18:1 + C19:0	0.13 ^c	0.17 ^{bc}	0.16 ^b	0.21 ^a	0.009	<0.01	0.13 ^c	0.16 ^b	0.17 ^b	0.20 ^a	0.008	<0.01
<i>cis</i> -16 C18:1 ⁴	0.06 ^b	0.08 ^{ab}	0.08 ^{ab}	0.11 ^a	0.010	<0.01	0.07 ^b	0.09 ^{ab}	0.09 ^b	0.13 ^a	0.012	<0.01
<i>trans</i> -6/7/8 C18:1	0.28	0.42	0.37	0.42	0.048	0.11	0.24 ^b	0.40 ^a	0.42 ^a	0.41 ^a	0.044	0.01
<i>trans</i> -9 C18:1	0.11	0.17	0.16	0.15	0.044	0.39	0.14	0.17	0.18	0.19	0.053	0.75
<i>trans</i> -10 C18:1	0.27 ^b	0.69 ^{ab}	0.41 ^{ab}	1.09 ^a	0.246	0.04	0.50 ^b	0.53 ^{ab}	0.35 ^b	1.02 ^a	0.148	0.01
<i>trans</i> -11 C18:1 (VA)	0.60 ^b	0.93 ^b	0.84 ^b	1.67 ^a	0.122	<0.01	0.43	0.83	0.94	0.82	0.191	0.14
<i>trans</i> -12 C18:1 ⁵	0.24 ^c	0.34 ^b	0.32 ^b	0.44 ^a	0.020	<0.01	0.22 ^c	0.33 ^b	0.34 ^b	0.40 ^a	0.015	<0.01
<i>trans</i> -16 C18:1 ⁶	0.24 ^b	0.30 ^{ab}	0.28 ^{ab}	0.37 ^a	0.033	<0.01	0.23 ^c	0.29 ^b	0.29 ^b	0.36 ^a	0.013	<0.01
<i>cis</i> -9, <i>cis</i> -12 C18:2 (LA)	1.16 ^b	1.12 ^b	1.12 ^b	1.31 ^a	0.061	<0.01	1.18 ^b	1.13 ^b	1.17 ^b	1.34 ^a	0.026	<0.01
<i>cis</i> -9, <i>trans</i> -13 C18:2 ⁷	0.10 ^c	0.14 ^b	0.13 ^b	0.20 ^a	0.009	<0.01	0.09 ^c	0.14 ^b	0.14 ^b	0.20 ^a	0.013	<0.01
<i>cis</i> -9, <i>trans</i> -11 C18:2 (CLA) ⁸	0.29 ^c	0.45 ^b	0.42 ^b	0.67 ^a	0.030	<0.01	0.28 ^c	0.40 ^b	0.42 ^b	0.57 ^a	0.036	<0.01
<i>trans</i> -11, <i>cis</i> -15 C18:2	0.05 ^b	0.08 ^b	0.09 ^b	0.19 ^a	0.016	<0.01	0.07 ^b	0.08 ^b	0.09 ^b	0.16 ^a	0.018	<0.01
<i>trans</i> -11, <i>trans</i> -15 C18:2	0.02 ^b	0.02 ^b	0.02 ^b	0.04 ^a	0.005	<0.01	0.01 ^b	0.02 ^b	0.02 ^b	0.03 ^a	0.006	0.02
C18:3n-3 ⁹ (ALA)	0.56 ^b	0.57 ^b	0.56 ^b	0.88 ^a	0.355	<0.01	0.54 ^b	0.55 ^b	0.60 ^b	0.77 ^a	0.021	<0.01
C20:0	0.36 ^a	0.34 ^{ab}	0.33 ^{ab}	0.31 ^b	0.017	0.03	0.33	0.32	0.32	0.31	0.014	0.16
C22:0	0.17 ^a	0.17 ^a	0.17 ^a	0.14 ^b	0.010	<0.01	0.17	0.16	0.17	0.16	0.006	0.41

^{a-c}Values with differing superscripts significantly differ ($P < 0.05$) from each other according to Tukey-corrected multiple comparisons.¹RL: raw lupines; EL1: lupines extruded at 115°C; EL2: lupines extruded at 135°C; EL3: lupines extruded at 150°C.²RLS: raw lupines with addition of reducing sugars; ELS1: lupines added with reducing sugars extruded at 115°C; ELS2: lupines added with reducing sugars extruded at 135°C; ELS3: lupines added with reducing sugars extruded at 150°C.³Includes minor proportions of *cis*-10 C18:1 and *trans*-15 C18:1.⁴Includes minor proportion of *cis*-9, *trans*-12 C18:2.⁵Includes minor proportion of *cis*-6 C18:1.⁶Includes minor proportion of *cis*-14 C18:1.⁷Includes minor proportions of C19:1 and *cis*-10, *trans*-14 C18:2.⁸Includes minor proportions of *trans*-7, *cis*-9 C18:2 and *trans*-8, *cis*-10 C18:2 isomers.⁹Includes minor proportion of *cis*-11 C20:1.

Table 7. Methane production and blood plasma metabolites in cows (n = 8 per experiment) fed raw or extruded white lupines without (Exp.1) or with the addition of reducing sugars (Exp.2); methane production was measured in a subset of cows (Exp.1: n = 4; Exp.2: n = 8)

Item ³	Lupines (Exp.1) ¹				SEM	P-value	Lupines + reducing sugars (Exp.2) ²					SEM	P-value
	RL	EL1	EL2	EL3			RLS	ELS1	ELS2	ELS3			
CH ₄ production, g/d	443	428	450	423	23.3	0.28	480	463	478	474	23.4	0.70	
CH ₄ yield, g/kg DMI	19.5	19.6	19.7	18.8	0.73	0.54	23.0	22.1	22.9	22.9	0.92	0.62	
CH ₄ intensity, g/kg ECM	15.9 ^a	15.9 ^a	15.6 ^a	14.0 ^b	0.70	<0.01	18.3	17.0	18.0	17.3	0.78	0.24	
CH ₄ energy, % of GE intake	5.79	5.82	5.83	5.55	0.22	0.50	6.78	6.54	6.78	6.72	0.27	0.64	
Plasma NEFA, mmol/L	0.104	0.156	0.103	0.173	0.053	0.96	0.132	0.149	0.103	0.136	0.020	0.20	
Plasma TAG, g/L	0.116	0.122	0.113	0.122	0.0098	0.70	0.117	0.122	0.124	0.137	0.0085	0.18	
Plasma total cholesterol, g/L	1.62	1.78	1.60	1.69	0.172	0.17	1.77	1.79	1.67	1.71	0.14	0.70	
Plasma acetate, mmol/L	0.982	0.948	0.922	0.911	0.105	0.87	0.951	1.039	1.034	0.975	0.106	0.81	

^{a,b}Values with differing superscripts significantly differ ($P < 0.05$) from each other according to Tukey-corrected multiple comparisons.

¹RL: raw lupines, EL1: lupines extruded at 115°C, EL2: lupines extruded at 135°C, EL3: lupines extruded at 150°C.

²RLS: raw lupines with addition of reducing sugars, ELS1: lupines added with reducing sugars extruded at 115°C, ELS2: lupines added with reducing sugars extruded at 135°C, ELS3: lupines added with reducing sugars extruded at 150°C.

³NEFA: nonesterified fatty acids, TAG: triacylglycerols; GE: gross energy.

phase for longer and potentially delay microbial access to embedded lipids.

In the present study, raw lupines exhibited relatively coarse and heterogeneous particle size distributions, whereas extrusion progressively reduced particle size as the extrusion temperature increased. The lupines extruded at 150°C showed the smallest Dv50 values and highest proportion of particles <1 mm, indicating a much finer and more uniformly fragmented material compared with lupines extruded at 115°C and 135°C. Such structural disruption is expected to increase lipid accessibility and therefore may have influenced the extent and timing of microbial access to FA and contributed to the accumulation of RBH intermediates observed with EL3.

Nevertheless, because rumen evacuations and digesta passage rate measurements were not performed, total FA pool sizes and biohydrogenation rates cannot be quantified from these data. The number of rumen-cannulated cows was limited by animal availability and ethical considerations inherent to intensive metabolism studies. Although no a priori power analysis was conducted, the sensitivity of the experimental design was evaluated based on observed variability. The present design allowed detection of minimal treatment differences of approximately 0.38, 1.26, 0.83, and 2.5 g/100 g of total FA for C18:3n-3, C18:2n-6, *trans*-11 C18:1, and C18:0, respectively, whereas smaller effects may have remained undetected. For these reasons, the ruminal FA results should be interpreted as descriptive snapshots rather than quantitative estimates of ruminal FA metabolism. To directly assess ruminal FA kinetics, future studies should incorporate measurements of digesta flow.

Effect of Lupine Extrusion on Milk Fatty Acid Profile

Extrusion of white lupines influenced the RBH of PUFA and, consequently, the milk FA profile. Across treatments, extrusion temperature also appeared to play a role in determining the availability of FA to rumen microbes, with the greatest effects consistently observed at 150°C compared with 115°C and 135°C. The addition of reducing sugars before extrusion did not markedly modify the impact of extrusion on lipid metabolism, as the effects on the milk FA profile were similar across both experiments.

As expected, the proportion of RBH intermediates in milk fat increased when cows were fed extruded lupines, regardless of sugar addition. In particular, *trans* MUFA proportions in milk fat increased with extrusion, with the highest proportions observed at 150°C (EL3 and ELS3). These results indicate that higher-temperature extrusion promoted incomplete biohydrogenation in the rumen, increasing the outflow of *trans* intermediates available for incorporation into milk fat. This agrees with previ-

ous studies in dairy cows fed thermomechanically treated oleaginous or oleo-proteaginous seeds such linseeds, soybeans, or rapeseed (Chouinard et al., 1997; Doreau et al., 2009; Dang Van et al., 2011).

The main CLA isomer in milk fat, *cis*-9,*trans*-11 C18:2 (rumenic acid), was significantly increased with all extruded lupine diets, with the highest proportions again observed with lupines extruded at 150°C. Rumenic acid in milk fat originates either from ruminal isomerization of LA or from endogenous synthesis in the mammary gland via Δ^9 -desaturase acting on its precursor, *trans*-11 C18:1 (Bernard et al., 2008). Because Δ^9 -desaturase activity did not seem to differ between dietary treatments, as indicated by a stable desaturation index (i.e., *cis*-9 C14:1-to-*trans*-11 C14:0), these increases likely originated from greater ruminal formation and subsequent escape of *trans*-11 C18:1, rather than from enhanced mammary desaturation. This interpretation is consistent with both our rumen digesta results and previous *in vitro*, *in situ* and *in vivo* findings showing that extrusion increases isomerization relative to hydrogenation in rapeseed or soybean products (Chouinard et al., 1997; Enjalbert et al., 2003; Troegeler-Meynadier et al., 2014).

However, differences in the apparent transfer rates of LA and ALA to milk fat were not fully aligned with the FA proportions measured in ruminal digesta. This discrepancy likely reflects the fact that these indicators capture different aspects of lipid metabolism. Ruminal FA profiles represent snapshot measurements of RBH at the time of sampling, whereas apparent transfer rates integrate several processes occurring between the rumen and the mammary gland, including extent of RBH, intestinal digestion and absorption of FA, and their transfer in plasma lipids and mammary uptake and secretion into milk fat. In addition, rumen samples were collected at discrete time points 12 h apart and may not fully reflect the diurnal dynamics of ruminal lipid metabolism. Consequently, apparent transfer rates may better represent the overall efficiency with which dietary PUFA reach milk fat over the course of the day, whereas ruminal FA proportions mainly reflect momentary ruminal conditions.

In contrast to results reported for extruded linseed (Akraim et al., 2007) and extruded soybean (Chouinard et al., 1997), extrusion of lupines did not substantially reduce the overall proportion of even-chain SFA in milk fat. The only exception was C16:0, which was lower with lupines extruded at 150°C. The lack of a broader reduction in SFA suggests that the degree of RBH inhibition or lipid protection achieved with lupines was less pronounced than with seeds richer in PUFA or oil. Although certain FA known to inhibit *de novo* synthesis in the mammary gland (e.g., *trans*-10 C18:1, *trans*-10,*cis*-12 CLA, LA and ALA) can be increased by thermomechanical processing (Harvatine et al., 2009), no reduction in

milk fat yield was observed in either experiment (Manzocchi et al., 2023). This indicates that extrusion of lupines, even at 150°C, did not induce milk fat depression, and this likely reflects the relatively moderate increases in inhibitory FA and the lack of major shifts toward *trans*-10 pathways in RBH.

Effect of Lupine Extrusion on Blood Metabolites

The absence of pre-feeding differences in plasma NEFA, TAG, cholesterol, and acetate supports the interpretation that extrusion with or without reducing sugars primarily modifies ruminal FA kinetics and the availability of C18 intermediates for mammary Δ^9 -desaturation, rather than altering basal systemic lipid metabolism. Indeed, this lack of treatment effect is consistent with the expectation that basal (pre-prandial) systemic lipid and acetate pools are tightly homeostatically regulated and only modestly affected by moderate changes in dietary lipid form when iso-energetic diets are fed. Methodologically, the pre-feeding blood sampling strategy reduced the sensitivity to detect transient postprandial differences in TAG and acetate. Future work should include post-prandial time-courses and kinetic measures to determine whether extrusion conditions alter circulating lipid fluxes despite similar basal concentrations.

Effect of Lupine Extrusion on Ruminal CH₄ Production

No effect of dietary treatment on daily CH₄ production was observed in either experiment. It should be noted, however, that despite a 2-wk measurement period, the number of AHC visits per cow was relatively low, which may have limited the sensitivity to detect small treatment-induced differences in CH₄ production.

Across all diets, ether extract averaged 4.1% $\pm$ 0.14% (mean $\pm$ SD) in the DM across all diets, which is below the commonly reported threshold of 6% to 7% EE in DM above which lipid supplementation may depress fiber digestion and reduce ruminal CH₄ production (Martin et al., 2020a). This is consistent with the absence of treatment effects on OM and NDF digestibility and on ruminal VFA proportions in the present experiments (Manzocchi et al., 2023). Similarly, Staerfl et al. (2012) reported no CH₄-mitigating effect of a corn silage-based diet containing 6% raw white lupine meal (DM basis) in growing bulls, although dietary EE and starch contents were not reported.

In contrast, Bryszak et al. (2020) predicted lower ruminal CH₄ production in dairy cows fed a diet containing 9.4% of narrow-leafed blue lupine meal (26.6 g EE/kg DM; starch content not reported) partially replacing soybean and rapeseed meal. Their prediction was based

on stoichiometric relationships among ruminal VFA proportions (Moss et al., 2000) and may reflect a more fermentable carbohydrate profile, including greater intake of starch and nonstarch polysaccharides. Diets richer in starch or rapidly fermentable carbohydrates generally yield lower ruminal CH₄ than fiber-rich diets (Bougouin et al., 2016), which may explain the discrepancy with the present results.

In the present study, extrusion-induced changes in the RBH did not appear to substantially modify ruminal H₂ sinks toward propionogenesis (Manzocchi et al., 2023) and methanogenesis. The contribution of RBH to total H₂ disposal is generally considered negligible (or at most, very limited) relative to methanogenesis (Beauchemin et al., 2022), which further supports the absence of a CH₄ response in our study. Finally, although daily CH₄ production and CH₄ yield (g/kg DMI) did not differ among diets, CH₄ intensity (g/kg ECM) was numerically lower with EL3 in Exp.1 due to the slightly higher ECM yield observed in cows fed this treatment (Manzocchi et al., 2023). Future studies including larger numbers of cows or herd-level measurements would help to confirm these results and improve the precision of treatment effects on ruminal CH₄ production.

CONCLUSIONS

Extrusion of white lupines, particularly at 150°C, modified the RBH of PUFA, increasing the transfer of vaccenic acid, CLA precursors, *trans* MUFA, and *cis*-9,*trans*-11 CLA into milk fat, irrespective of reducing sugar pretreatment. In contrast, extrusion did not affect blood metabolites and daily CH₄ production or CH₄ yield, and the lower CH₄ intensity observed with lupines extruded at 150°C resulted solely from a higher milk yield.

NOTES

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Nonstandard abbreviations used: AHC = automated head-chamber; ALA = α -linolenic acid; Dv50 = lowest diameter at 50% of the cumulative volume distribution; EE = ether extract; EL1 = lupine seeds extruded at 115°C; EL2 = lupine seeds extruded at 135°C; EL3 = lupine seeds extruded at 150°C; ELS1 = lupine seeds with added reducing sugars extruded at 115°C; ELS2 = lupine seeds with added reducing sugars extruded at 135°C; ELS3 = lupine seeds with added reducing sugars extruded at 150°C; Exp.1 = experiment 1; Exp.2 = experiment 2; FA = fatty acid; GE = gross energy; LA = linoleic acid; NEFA = nonesterified fatty acid; NI = not identified; PDI = protein truly digestible in the intestine; RBH = ruminal biohydrogenation; RPB = rumen protein balance; RL = raw white lupine seeds; RLS = raw lupine seeds with added reducing sugars; TAG = triacylglycerols; TC = total cholesterol; UFL = Unité Fourragère Lait.

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