Animal (2007), **1:6**, pp 911–921 © The Animal Consortium 2007
doi: 10.1017/S1751731107000316

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Effect of botanical composition of silages on rumen fatty acid metabolism and fatty acid composition in *longissimus* muscle and subcutaneous fat of lambs

M. Lourenço, S. De Smet, K. Raes[†] and V. Fievez[‡]

Laboratory for Animal Nutrition and Animal Product Quality, Department of Animal Production, Ghent University, Proefhoevestraat 10, 9090 Melle, Belgium

(Received 20 November 2006; Accepted 20 March 2007)

To study the effect of feeding silages with different botanical composition, on rumen and lamb fat, 30 male lambs were assigned to five different silage groups for 11 weeks: botanically diverse silage (BDS); white clover silage (WCS); red clover silage (RCS), intensive English ryegrass silage (IRS) and crushed linseed and maize silage (MSL). Besides the silages, animals received organic wheat and barley and the MSL group additionally received bicarbonate (15 g/day). Silages were sampled when the bales were opened and analysed for fatty acid (FA) content and chemical composition. At slaughter, ruminal contents were sampled and 24 h after slaughter, longissimus *muscle and subcutaneous (SC) fat were sampled. All samples were analysed for FA composition. The MSL group ingested the highest amount of FA (35.8 g/day* v. *13.5, 19.4, 17.2 and 30.4 g/day for MSL* v. *BDS, WCS, RCS and IRS, respectively) and the sum of the major polyunsaturated FA, C18:2 n-6 and C18:3 n-3, was similar for groups BDS, WCS, RCS and MSL (61.3 g/100 g, 62.3 g/100 g, 62.3 g/100 g, 63.7 g/100 g of FA methylesters (FAME), respectively), while group IRS ingested higher proportions of these FA (74.5 g/100 g of FAME). Rumen data showed that animals fed BDS presented higher proportions of biohydrogenation intermediates, particularly C18:1 t11 and CLA c9t11, suggesting partial inhibition of rumen biohydrogenation. In the MSL group, the content of C18:3 n-3 in the rumen was highest, most probably due to reduced lipolysis and hence biohydrogenation through the combined effect of esterified C18:3 n-3 and seed protection. Additionally, C18:3 n-3 proportions were higher in rumen contents of RCS animals compared with WCS animals, which could be due to the activity of the polyphenol oxidase enzyme in the RC silages. Proportions of C18:3 n-3 were similar between treatments both for SC and intramuscular (IM) fat, whereas CLA c9t11 content was higher in the SC fat of BDS animals and lower in the IM fat of IRS animals compared with the other forage groups. No differences were found for C20:4 n-6, C20:5 n-3, C22:5 n-3 and C22:6 n-3 in the IM fat of the animals. Nevertheless, indices for desaturation and elongation activity in muscle of BDS animals suggest some stimulation of the first three steps of desaturation and elongation (Δ6-desaturase, elongase and Δ5-desaturase) of long-chain FA.*

Keywords: biohydrogenation, botanical composition, fatty acids, metabolism, silage

Introduction

Some recent studies have demonstrated that the botanical composition of grazed pastures affects the fatty acid (FA) profile of milk (Collomb *et al.*, 2002; Žan *et al.*, 2006) and meat (Ådnøy *et al.*, 2005; Lourenço *et al.*, 2007). Moreover, simultaneous determination of rumen, subcutaneous (SC) and intramuscular (IM) FA profiles revealed some of the changes in the tissue FA profile to be related to modifications of the rumen and tissue FA metabolism. Indeed, grazing a botanically diverse pasture compared with an intensive ryegrass pasture resulted in an accumulation of biohydrogenation intermediates in the rumen and in an increase of long-chain polyunsaturated FA (PUFA) in IM fat (Lourenço *et al.*, 2007), suggesting a partial inhibition of rumen biohydrogenation and a stimulation of desaturation and elongation of PUFA. Within the same context, former work at our laboratory (Lourenço *et al.*, 2005b) revealed higher milk conjugated linoleic acid (CLA) proportions when dairy cows were fed a mixture of silage richer in herb species than cows fed intensively managed ryegrass silage. Other studies (Dewhurst *et al.*, 2003a and

[†] Present address: EnBiChem, Department PIH, University College of West-Flanders, Graaf Karel de Goedelaan 5, 8500 Kortrijk, Belgium.
[‡] E-mail: veerle.fievez@UGent.be

2003b; Lee *et al*., 2003) also reported a reduced rumen biohydrogenation of FA from clover-rich silages compared with other alfalfa and grass silages. Changes in rumen FA metabolism might be related to the presence of specific herbs in botanically diverse pastures, as they are reported to contain metabolites with antimicrobial properties (Wallace, 2004).

The effect of the botanical composition of silages on SC and IM FA composition has not been studied in depth. Thus, the objectives of this study were to describe the IM and SC FA composition in relation to (i) feeding intensive ryegrass *v.* clover *v.* botanically diverse silage (BDS); (ii) white *v.* red clover silage (RCS) feeding and (iii) C18:3 n-3 supply from forage silages *v.* linseed. Moreover, rumen FA composition and muscle FA indices were used to assess some indicators for rumen and muscle FA metabolism.

Material and methods

Animals

Thirty male lambs of similar genetic background ('Vlaams Kuddeschaap', a typical 'herding' sheep breed), born from yearling ewes and originating from an organic farm (Berendrecht, Belgium) were used. Before the beginning of the trial, lambs were grazing with their mothers on pastures of the organic farm of origin. At weaning, animals were assigned based on their live weight and age to one of the five different groups (six lambs per group), i.e. a group fed botanically diverse silage (BDS), white clover silage (WCS), red clover silage (RCS), intensive English ryegrass silage (IRS) or maize silage and crushed linseed (MSL). The average age and live weight at the onset of the experimental period was 118±8 days and 29.6±3.6 kg respectively, and did not differ significantly between groups. Animals of the same group were divided into two pens (three animals per pen).

Feeding and diets

The experiment lasted 11 weeks (5 July 2005 until 19 September 2005). Animals were fed in the morning at 0800 h (700 g/kg dry matter (DM) of silage and 300 g/kg DM of a mixture of wheat and barley, separately). The amount of feed was adjusted per pen every 15 days to meet net energy (Voedereenheid Vleesproductie Intensief (VEVI), Van Es, 1978; Centraal Veevoederbureau (CVB), 2004) and protein (Darmverteerbaar Eiwit (DVE), Tamminga *et al*., 1994; CVB, 2004) requirements in accordance with the average growth rate of the three animals per pen. Essential minerals (sodium (270 g/kg), calcium (60 g/kg), phosphorus (2 g/kg) and magnesium (1 g/kg)) and micronutrients (zinc (18 000 mg/kg), manganese (2 000 mg/kg), iodine (100 mg/kg), cobalt (40 mg/kg) and selenium (10 mg/kg)) were provided by a mineral block for sheep (Timac Potasco, Belgium).

Red clover, white clover and BDSs were provided in bales of approximately 270 kg. These silages were from natural grassland pastures situated at the farm of origin (Berendrecht, Belgium, 51°20N/04°28E, 14 m a.s.l.) and without any type of fertilisation. These silages were baled during the summer of 2004 (at the second and third cuts). The silages were wilted for 48 h and no inoculum was used. Silage bales of intensive English ryegrass were made from a pasture with *circa* 70% of *Lolium perenne* – English ryegrass (the other 30% were mainly *Bromus hordeaceus* – soft brome – and *Lolium multiflorum* – Italian ryegrass) and fertilised with organic manure (30 to 40 ton/ha cow manure) at the end of February 2005, with 200 kg/ha of ammonium nitrate (25% N) on 21 March 2005 and with organic manure (25 ton/ha pig manure) after the first cut at the end of April 2005. Maize silage was produced from a maize crop fertilised with 30 to 40 ton/ha of cow or pig manure and 210 kg/ha 30% N – 10% P_2O_5. Both the English ryegrass pasture and the maize crop were situated at the experimental farm of Ghent University at Melle, Belgium (50°59′N/03°49′E, 11 m a.s.l.).

In addition to the silages, all groups received organic ground wheat and barley grains. The ratio of wheat and barley was adapted during the experimental period to meet energy and protein requirements for growth. Animals in group MSL received extra crushed linseed in order to provide C18:3 n-3 in the range of the supply of the forage silages. The MSL group received also 15 g of sodium bicarbonate daily, in order to prevent rumen acidosis. Animals had free access to water.

Measurements and sampling

The silage portions were prepared per pen every time a bale was opened (on average every 9 days for the groups BDS, WCS and RCS, and weekly for the IRS group). Maize silage portions were prepared weekly from the silo. All daily portions of silage for the different groups were kept in the fridge at 4°C until fed to the animals. Wheat, barley and crushed linseed portions were prepared every 3 days. Leftovers of silage were recovered daily and weighed to assess the average intake per pen. There were no leftovers of grains and linseed. The amount of silage and grains distributed and the barley/wheat ratio were adjusted according to the average weight and growth rate per pen in order to provide 110% of the energy (VEVI, Van Es, 1978; CVB, 2004) and protein (DVE, Tamminga *et al*., 1994; CVB, 2004) requirements.

All silages were sampled for FA analysis, DM determination and chemical composition at time of weighing. Wheat, barley and linseed were sampled every 4 weeks for FA analysis and chemical composition. Samples were taken directly to the lab where FA extraction and DM determination were performed immediately. Samples for chemical composition analysis were stored at −20°C.

At the end of the experimental period, the lambs were transported to a private abattoir (Ronse, Belgium) without prior fasting and slaughtered according to conventional practise. Ruminal (1 l) contents were sampled into plastic

https://doi.org/10.1017/S1751731107000316 Published online by Cambridge University Press

pots after thorough mixing, and kept refrigerated until arrival in the laboratory. To ensure correct sampling, the pH of rumen contents was measured at three different locations. Rumen subsamples (25 ml) were prepared for volatile fatty acid (VFA) analysis, as soon as they arrived in the laboratory. Samples were acidified with 0.5 ml of phosphoric/formic acid (10/1, vol/vol) and centrifuged for 15 min at 31 000 × **g**. The supernatant was recovered and 1 ml was transferred to vials and analysed by gas chromatography (Schimadzu GC-14A, Belgium) according to Van Nevel and Demeyer (1977). The rest of the rumen samples were freeze-dried and kept at −20°C until analysis of FA.

Meat and SC fat samples were taken 24 h after slaughter from chilled carcasses (4°C). Meat samples were taken from the *m. longissimus thoracis* from the left side of the carcass (between T7 and T8). Meat and SC fat samples were stored vacuum packed at −20°C until FA analysis. Meat samples were trimmed of external fat so that only IM fat was extracted and analysed.

Chemical composition analysis

Silage samples for chemical composition determination were freeze-dried, ground through a 1.5-mm mesh (Brabander, Duisburg, Germany) and further pooled per 4 weeks. Wheat, barley and crushed linseed were finely (0.5 to 1 mm) ground (Grindomix GM 200, Retsch, Germany) and further analysed. Chemical composition analysis consisted of determination of crude protein, according to the Kjeldahl method (European Community, 1993), ADF and NDF using the method of Van Soest *et al*. (1991), and crude fat with the Soxhlet method (International Organisation for Standardisation, 1973). Results are presented in Table 1.

Fatty acid analysis

Extraction. FAs of all silage samples were extracted in duplicate with chloroform/methanol (2/1, vol/vol) (C/M), as described by Lourenço *et al*. (2007). Briefly, 5 g of fresh material were cut into 1 cm strips and homogenised for 1 min (Ultra-Turrax T25, IKA-Labortechnik, Belgium). The endogenous water was determined (105°C for 4 h) in order to adjust the ratio of chloroform/methanol/water to 8/4/3 (vol/vol/vol). In all samples, 40 ml of C/M (2/1, vol/vol) was added, and 10 mg of nonadecanoic acid (C19:0; Sigma, Belgium) used as internal standard and samples were extracted overnight. The next morning, samples were centrifuged at 1821 × **g** for 10 min and the C/M layer was recovered. In the second and third extraction step, 30 and 20 ml of C/M (2/1, vol/vol) respectively, were added and the samples were centrifuged at 1821 × **g** for 10 min for every extraction step. The extracts were combined and washed once with distilled water and the C/M layer recovered. Finally, the extracts were brought to a final volume of 100 ml with C/M (2/1, vol/vol).

Wheat, barley and linseed (finely ground, 0.5 to 1 mm (Grindomix GM 200) and rumen (freeze-dried and finely ground as for wheat, barley and linseed) samples were analysed in duplicate for FA as described by Lourenço *et al*. (2005b). Briefly, 2.5 g of sample was extracted overnight with 30 ml of C/M (2/1, vol/vol), 20 ml of distilled water and 10 mg of nonadecanoic acid (C19:0) as internal standard. The samples were then centrifuged at 1821 × **g** for 10 min and the C/M layer recovered. This procedure was repeated twice, adding 25 ml of C/M (2/1, vol/vol) in the second and 20 ml in the third extraction step. Finally, samples were washed with distilled water and the C/M layer was recovered. Extracts were brought to a final volume of 100 ml with C/M (2/1, vol/vol).

Meat samples were extracted in duplicate as described by Raes *et al*. (2001). Briefly, 5 g of meat was homogenised for 30 s (Ultra-Turrax T25, IKA-Labortechnik, Belgium) and extracted overnight with 30 ml of C/M (2/1, vol/vol) and 3 ml of butylated hydroxytoluene (BHT) in chloroform (0.1% w/vol). Samples were then filtered (Fiorini, S.A.) and the filtrate collected. The filter was washed twice with 10 ml of C/M (2/1, vol/vol). The filtrate was then transferred to the extraction tubes and 15 ml of distilled water was added. Samples were centrifuged at 1821 × **g** for 10 min and the C/M layer recovered and evaporated with a rotavapor (Laborota 4000 WB, Germany) at 40°C. The dry residue was then re-suspended in 10 ml of C/M (2/1, vol/vol).

SC fat samples (1 g) were extracted using a procedure similar to that described above for FA extraction of meat

Table 1 *Chemical composition of the five different silages (n = 3) and of the grains (n = 2) given to the animals*[†]

	DM (g/kg)	NDF (g/kg DM)	ADF (g/kg DM)	Fat (g/kg DM)	Protein (g/kg DM)
Silages					
Botanically diverse	668	579	383	26.4	94.4
White clover	609	530	348	32.5	113
Red clover	521	523	366	30.3	106
Intensive ryegrass	380	393	259	40.2	129
Maize	351	471	250	40.5	71.6
Grains					
Wheat	–	314	58.5	46.0	111
Barley	–	263	70.6	30.8	86.0
Linseed	–	336	175	394	186

[†]Abbreviations are: DM = dry matter; NDF = neutral-detergent fibre; ADF = acid-detergent fibre.

https://doi.org/10.1017/S1751731107000316 Published online by Cambridge University Press

(Raes *et al.*, 2001); however, the bottom layer was recovered into volumetric flasks after washing with distilled water and was brought to a final volume of 100 ml with C/M (2/1, vol/vol).

Methylation. For methylation of IM and SC lipids, 2 ml of extract was taken and 1 ml of nonadecanoic acid (2 mg/ml; C19:0; Sigma) was added. For methylation of silage, wheat, barley, linseed and rumen lipids, 10 ml of extract was used. Samples were methylated at 50°C with NaOH in methanol (0.5 mol/l) followed by HCl/methanol (1/1, vol/vol) according to Raes *et al.* (2001).

Gas chromatography (GC). Fatty acid methylesters (FAME) were analysed on a Hewlett-Packard 6890 gas chromatograph (Hewlett-Packard Co., Belgium) with a CP-Sil88 column for FAME (100 m × 0.25 mm × 0.2 μm; Chrompack Inc., The Netherlands). For more detailed information about the GC conditions for analysis of silage, wheat, barley, linseed, rumen, IM and SC fat samples, we refer to Raes *et al.* (2004b). Separation of the FA C16:1 *t*9 and *iso* C17:0 was not possible due to the status of the GC column. Conjugated linoleic acid *cis–cis* (CLAcc) isomers and CLA *trans–trans* (CLAtt) isomers are reported as the sum of all CLA isomers with two *cis* or *trans* double bounds, respectively, as with the GC method used it was not possible to separate all CLAcc and all CLAtt isomers.

Statistics

A one-way ANOVA was used to compare the feed FA content and composition of each group and to evaluate the effect of the different diets on rumen, IM and SC fat FA and rumen VFA, according to $Y_i = \mu + B_i + \varepsilon_i$, where μ is the overall mean, B_i the effect of the different silages and ε_i the residual error. Five orthogonal contrasts were applied: (1) MSL diet *v.* the four other diets to compare the supply of forage C18:3 n-3 (mainly unesterified) *v.* linseed C18:3 n-3 (mainly in triacylglycerols); (2) BDS diet *v.* WCS + RCS diets to compare botanical diversity with clover-rich diets; (3) BDS diet *v.* IRS diet; (4) WCS + RCS diets *v.* IRS diet to compare clover-rich diets with ryegrass; (5) WCS diet *v.* RCS diet.

Principal component analysis (PCA), based on the correlation matrix, was conducted to determine components which account for most of the total variation in odd- and branched-chain FA (OBCFA). Each object (animal × treatment, $n = 30$) was considered to be a data vector of 11 variables (*iso* C13:0, *anteiso* C13:0, C13:0, *iso* C14:0, *iso* C15:0, *anteiso* C15:0, C15:0, *iso* C16:0, *anteiso* C17:0, C17:0 and C17:1 c9 all expressed as g/100 g of FAME). The principal component scores are presented in a scatter plot to evaluate grouping of treatments. Statistical analyses were performed using Statistical Packages for the Social Sciences (2003).

Results

Live-weight gain of the animals in group BDS tended to be lower compared with the animals of the other groups, with an average live weight at slaughter of 33.0 kg for the BDS group compared with 37.7, 35.1, 40.8 and 37.9 for the WCS, RCS, IRS and MSL groups, respectively.

Diets

In Table 2, total average individual FA and proportions of FA ingested by the animals is presented. For the first four diets, 96% of all FA is reported, whereas for diet MSL, 98% of all FA is reported. Animals of the MSL group ingested the highest total amount of FA, followed by animals of group IRS, with animals fed the BDS diet ingesting the lowest amount. Proportions of C16:0 in the MSL diet were significantly lower than for the four forage diets. Proportions of C18:2 n-6 were significantly higher for the group MSL, followed by BDS, WCS and RCS groups and were lowest for the IRS diet. On the other hand, proportions of C18:3 n-3 were lowest for the MSL group and highest for the IRS diet. Nevertheless, in terms of C18:3 n-3 intake, the MSL group was intermediate (9.67 g/day) between the IRS (15.1 g/day) and the clover diets (7.04 and 6.45 g/day, respectively), whereas the BDS group ingested the lowest C18:3 n-3 amount (4.48 g/day).

Proportions of PUFA (C18:2 n-6+C18:3 n-3) were similar for diets BDS, WCS, RCS and MSL (61.3, 62.3, 62.3 and 63.7 g/100 g of FAME for BDS, WCS, RCS and MSL respectively) while this was higher for diet IRS (74.5 g/100 g of FAME).

Fatty acid composition of rumen contents

Total rumen concentrations of VFA, proportions of individual VFA and VFA ratios are presented in Table 3. Animals in the MSL group presented a significantly lower proportion of acetate and a significantly higher proportion of propionate compared with the forage silage fed groups. Proportions of butyrate did not differ significantly between the five groups, with only a trend ($P = 0.060$) for animals in BDS group to show higher butyrate proportions than animals in the IRS group. Lambs in the IRS group tended to have higher valerate proportions compared with lambs in the BDS group ($P = 0.084$) and in the WCS + RCS groups ($P = 0.083$).

Total FA concentration and proportions of FA of rumen contents are presented in Table 4. For rumen contents, the proportions of C18-FA are also expressed relative to the sum of all C18-FA identified, as this allows a better evaluation of rumen hydrogenation when dietary supply of C18-FA differs (Chow *et al.*, 2004). Rumen contents of MSL animals clearly had the highest amount of total FA, followed by animals in the IRS group, WCS and RCS group, whereas total rumen FA content was lowest for animals of the BDS group. Proportions of C18:2 n-6 were significantly lower in rumen contents of IRS animals compared with the BDS group and only a trend was found for lower proportions of C18:2 n-6 for the IRS group compared with the WCS + RCS groups ($P = 0.088$). C18:3 n-3 proportions were higher in the rumen contents of the MSL group than in forage silage fed groups and were lower in animals of the BDS group than in animals of WCS + RCS groups. Further, lambs fed WCS tended ($P = 0.077$) to have lower proportions of C18:3 n-3 in their rumen contents than lambs fed RCS.

https://doi.org/10.1017/S1751731107000316 Published online by Cambridge University Press

Table 2 *Total average individual DM (kg/day) and FA (g/day) intakes and proportions of FA (g/100 g FAME) ingested by the animals fed the five different diets* (n = *11*)

							Significance†				
	BDS	WCS	RCS	IRS	MSL	s.e.	1	2	3	4	5
Total DM intake	1.02	1.24	1.11	1.16	1.29	0.075	T	n.s.	n.s.	n.s.	n.s.
Total FA intake	13.5	19.4	17.2	30.4	35.8	3.10	***	***	***	***	n.s.
C12:0	0.232	0.139	0.288	0.162	0.075	0.020	***	n.s.	*	*	***
C14:0	0.500	0.462	0.679	0.465	0.210	0.030	***	T	n.s.	**	***
C16:0	18.5	17.7	17.7	14.9	11.5	0.237	***	**	***	***	n.s.
C16:1 *c*9	1.21	1.33	1.23	1.34	0.094	0.065	***	n.s.	n.s.	n.s.	n.s.
C17:0	0.283	0.270	0.275	0.158	0.129	0.009	***	n.s.	***	***	n.s.
C18:0	2.57	2.50	2.68	1.79	2.99	0.097	***	n.s.	***	***	n.s.
C18:1 *c*9	6.50	5.90	5.78	3.72	17.6	0.329	***	*	***	***	n.s.
C18:2 n-6	28.1	26.0	24.8	18.6	36.7	0.776	***	**	***	***	n.s.
C18:3 n-3	33.2	36.3	37.5	49.7	27.0	1.28	***	*	***	***	n.s.
Total C18	71.6	71.7	71.4	74.5	85.2	0.417	***	n.s.	***	***	n.s.
Total OBCFA‡	3.65	3.84	3.89	4.01	0.687	0.129	***	n.s.	*	n.s.	n.s.
Phytanic acid	0.077	0.071	0.009	0.019	0.151	0.011	***	**	***	n.s.	***

Abbreviations are: DM = dry matter; FA: fatty acid; FAME: FA methylesters; BDS = botanically diverse silage; WCS = white clover silage; RCS = red clover silage; IRS = intensive English ryegrass silage; MSL = crushed linseed and maize silage; s.e.: standard error; OBFCA: odd- and branched-chain fatty acid.
†T = trend ($0.1 < P < 0.05$); * = $0.05 < P < 0.01$; ** = $0.01 < P < 0.001$; *** = $P < 0.001$; n.s. = non-significant; 1 = orthogonal contrast MSL *v.* forages; 2 = orthogonal contrast BDS *v.* clover diets; 3 = orthogonal contrast BDS *v.* IRS; 4 = orthogonal contrast clover diets *v.* IRS; 5 = orthogonal contrast WCS *v.* RCS.
‡Total OBCFA – sum of all odd- and branched-chain fatty acids: C13:0 *iso*; C13;0 *anteiso*; C13:0; C14:0 *iso*; C15:0 *iso*; C15:0 *anteiso*; C15:0; C16:0 *iso*; C17:0 *iso*; C17:0 *anteiso*; C17:0; C17:1 *c*9.

Table 3 *Total VFA concentration (mmol/l) and relative proportions of VFA (mmol/mol total VFA) in the rumen of animals fed the five different diets* (n = *6*)

							Significance†				
	BDS	WCS	RCS	IRS	MSL	s.e.	1	2	3	4	5
Total	128	136	147	120	112	9.00	*	n.s.	n.s.	T	n.s.
Relative proportions of VFA											
Acetate	698	720	722	685	592	22.1	***	n.s.	n.s.	n.s.	n.s.
Propionate	137	134	137	168	223	18.7	**	n.s.	n.s.	n.s.	n.s.
*Iso*butyrate	8.62	7.62	7.42	8.62	31.0	2.99	***	n.s.	n.s.	n.s.	n.s.
Butyrate	140	125	115	111	124	10.5	n.s.	n.s.	T	n.s.	n.s.
*Iso*valerate	7.84	5.52	7.04	6.84	14.7	1.55	***	n.s.	n.s.	n.s.	n.s.
Valerate	9.16	8.92	12.4	20.5	15.6	4.46	n.s.	n.s.	T	T	n.s.
Rumen pH	5.67	5.49	5.56	5.42	5.84	0.112	*	n.s.	n.s.	n.s.	n.s.

Abbreviations are: VFA = volatile fatty acid; BDS = botanically diverse silage; WCS = white clover silage; RCS = red clover silage; IRS = intensive English ryegrass silage; MSL = crushed linseed and maize silage; s.e.: standard error.
†T = trend ($0.1 < P < 0.05$); * = $0.05 < P < 0.01$; ** = $0.01 < P < 0.001$; *** = $P < 0.001$; n.s. = non-significant; 1 = orthogonal contrast MSL *v.* forages; 2 = orthogonal contrast BDS *v.* clover diets; 3 = orthogonal contrast BDS *v.* IRS; 4 = orthogonal contrast clover diets *v.* IRS; 5 = orthogonal contrast WCS *v.* RCS.

Rumen contents of BDS animals presented a higher sum of the proportions of biohydrogenation intermediates (C18:1 *t*11, C18:1 *t*15, C18:1 *c*15, C18:2 *t*11*c*15, CLA *c*9*t*11 and C18:3 *c*9*t*11*c*15) of the major rumen biohydrogenation pathways of C18:2 n-6 and C18:3 n-3 than the other groups (4.53 g/100 g of FAME *v.* 3.88, 4.30, 3.60 and 3.44 g/100 g of FAME for BDS *v.* WCS, RCS, IRS and MSL groups, respectively). This is mainly due to the isomers C18:1 *t*11 and CLA *c*9*t*11. In addition, rumen contents of animals of the WCS and RCS groups contained higher proportions of C18:2 *t*11*c*15 compared with BDS and IRS groups and RCS animals had higher C18:1 *c*15 and C18:1 *t*15 proportions than WCS animals. Rumen contents of animals of groups IRS and MSL had significantly higher proportions of C18:0 and lower accumulation of the major biohydrogenation intermediates compared with the other groups, except C18:1 *c*15 and C18:1 *t*15 proportions which were highest for the IRS group. The differences seen between groups remain when these intermediates are expressed relative to the sum of all C18-FA (Table 4). On the other hand, concerning intermediates of secondary biohydrogenation pathways, rumen contents of group MSL contained the

https://doi.org/10.1017/S1751731107000316 Published online by Cambridge University Press

Table 4 *Total concentration (mg/g dry matter) and proportions of individual FAs (g/100 g FAME) in rumen contents of animals fed the five different diets (n = 6)*

FAs	BDS	WCS	RCS	IRS	MSL	s.e.	Significance†				
							1	2	3	4	5
Total	24.6	33.6	33.5	50.2	75.1	3.54	***	*	***	**	n.s.
C12:0	0.186	0.175	0.166	0.117	0.122	0.028	n.s.	n.s.	T	n.s.	n.s.
C14:0	0.834	0.703	0.635	0.492	0.188	0.052	***	*	***	*	n.s.
C16:0	22.5	19.0	18.8	14.3	11.6	0.511	***	***	***	***	n.s.
C18:0	40.3	48.4	44.1	53.8	59.0	2.17	***	*	***	*	n.s.
C18:1 *t*9	0.098	0.115	0.156	0.163	0.139	0.017	n.s.	T	*	n.s.	n.s.
C18:1*t*10	0.271	0.315	0.443	0.489	0.926	0.122	***	n.s.	n.s.	n.s.	n.s.
C18:1*t*11	2.51	2.01	2.20	1.05	1.63	0.278	n.s.	n.s.	**	**	n.s.
C18:1*t*15	0.309	0.573	0.978	1.77	0.904	0.104	n.s.	**	***	***	*
C18:1*c*9	5.70	4.05	4.64	5.03	5.81	0.467	T	*	n.s.	n.s.	n.s.
C18:1*c*15	0.070	0.040	0.073	0.109	0.068	0.013	n.s.	n.s.	T	**	T
C18:2 *t*11*c*15	0.185	0.294	0.316	0.179	0.227	0.037	n.s.	*	n.s.	*	n.s.
C18:2 n-6	5.91	4.29	4.97	2.92	5.46	0.753	n.s.	n.s.	*	T	n.s.
CLA*c*9*t*11	1.31	0.788	0.589	0.330	0.517	0.123	T	***	***	*	n.s.
CLA*c*11*t*13	0.159	0.105	0.123	0.045	0.052	0.043	n.s.	n.s.	T	n.s.	n.s.
CLA*t*10*c*12	0.137	0.077	0.074	0.053	0.027	0.018	**	*	**	n.s.	n.s.
CLAcc	0.056	0.088	0.067	0.070	0.023	0.024	T	n.s.	n.s.	n.s.	n.s.
CLAtt	0.097	0.070	0.113	0.381	0.148	0.032	n.s.	n.s.	***	***	n.s.
C18:3*c*9*t*11*c*15	0.141	0.171	0.148	0.158	0.091	0.077	n.s.	n.s.	n.s.	n.s.	n.s.
C18:3 n-3	1.00	1.62	2.61	1.76	4.87	0.386	***	*	n.s.	n.s.	T
Total C18	59.7	64.3	63.7	71.2	82.4	0.981	***	**	***	***	n.s.
Total MUFA‡	13.2	11.9	14.3	15.1	12.6	1.12	n.s.	n.s.	n.s.	n.s.	n.s.
Total OBCFA‡	8.00	6.27	6.65	4.99	2.94	0.371	***	**	***	**	n.s.
C16:1*t*9+iso C17:0	1.24	0.726	0.629	0.430	0.424	0.061	***	***	***	**	n.s.
iso C14:0	0.170	0.177	0.253	0.239	0.019	0.029	***	T	n.s.	T	n.s.
C18 FAs as % of total C18											
C18:0	67.5	75.2	69.1	75.5	71.5	2.54	n.s.	n.s.	*	n.s.	T
C18:1 t9	0.164	0.179	0.246	0.230	0.170	0.122	n.s.	n.s.	T	n.s.	T
C18:1 *t*10	0.449	0.490	0.697	0.688	1.13	0.151	**	n.s.	n.s.	n.s.	n.s.
C18:1 *t*11	4.23	3.12	3.46	1.48	1.99	0.396	*	T	***	**	n.s.
C18:1 *t*15	0.514	0.894	1.54	2.49	1.10	0.150	n.s.	**	***	***	**
C18:1 *c*9	9.62	6.31	7.30	7.07	7.08	0.689	n.s.	**	*	n.s.	n.s.
C18:1 *c*15	0.118	0.063	0.116	0.153	0.082	0.018	n.s.	n.s.	n.s.	*	*
C18:2 *t*11*c*15	0.320	0.457	0.499	0.252	0.274	0.058	n.s.	*	n.s.	**	n.s.
C18:2 n-6	9.74	6.68	7.78	4.10	6.66	1.06	n.s.	T	**	*	n.s.
CLA *c*9*t*11	2.22	1.23	0.930	0.465	0.628	0.204	*	***	***	*	n.s.
CLA *c*11*t*13	0.282	0.164	0.194	0.063	0.063	0.073	n.s.	n.s.	T	n.s.	n.s.
CLA *t*10*c*12	0.236	0.121	0.116	0.074	0.033	0.033	**	**	**	n.s.	n.s.
CLA cc	0.095	0.137	0.106	0.099	0.029	0.039	T	n.s.	n.s.	n.s.	n.s.
CLA tt	0.166	0.108	0.179	0.536	0.178	0.044	n.s.	n.s.	***	***	n.s.
C18:3 *c*9*t*11*c*15	0.231	0.261	0.228	0.219	0.109	0.112	n.s.	n.s.	n.s.	n.s.	n.s.
C18:3 n-3	1.69	2.52	4.11	2.46	5.91	0.479	***	**	n.s.	n.s.	*

Abbreviations are: FA: fatty acid; FAME: FA methylesters; BDS = botanically diverse silage; WCS = white clover silage; RCS = red clover silage; IRS = intensive English ryegrass silage; MSL =crushed linseed and maize silage; s.e.: standard error; MUFA = monounsaturated fatty acid; OBFCA: odd- and branched-chain fatty acid.

†T = trend ($0.1 < P < 0.05$); * = $0.05 < P < 0.01$; ** = $0.01 < P < 0.001$; *** = $P < 0.001$; n.s. = non-significant; 1 = orthogonal contrast linseed *v.* forages; 2 = orthogonal contrast BDS *v.* clover diets; 3 = orthogonal contrast BDS *v.* IRS; 4 = orthogonal contrast clover diets *v.* IRS; 5 = orthogonal contrast WCS *v.* RCS.

‡Total MUFA = sum of monounsaturated fatty acids: C14:1 *c*9, C15:1 *c*9, C16:1 *t*9, C16:1 *c*9, C17:1 *c*9, C18:1 *t*6-*t*8, C18:1 *t*9, C18:1 *t*10, C18:1 *t*11, C18:1 *t*12-t14, C18:1 *t*15, C18:1 *c*9, C18:1 *c*10, C18:1 *c*11, C18:1 *c*12, C18:1 *c*14, C18:1 *c*15 and C20:1 *c*9. Total OBCFA = sum of all odd- and branched-chain fatty acids: C13:0 *iso*; C13:0 *anteiso*; C13:0; C14:0 *iso*; C15:0 *iso*; C15:0 *anteiso*; C15:0; C16:0 *iso*; C17:0 *iso*; C17:0 *anteiso*; C17:0; C17:1 c9.

highest proportions of C18:1 *t*10 compared with the forage silage fed groups, whereas BDS animals had unexpectedly significantly higher CLA *t*10*c*12 proportions in their rumen contents compared with the other groups.

Subcutaneous and intramuscular fatty acid composition

The FA acid pattern of the SC fat was a partial reflection of what was found in the rumen (Table 5). Total concentration of FA in the SC fat was similar between groups as well as

https://doi.org/10.1017/S1751731107000316 Published online by Cambridge University Press

Table 5 *Total concentration (mg/g fat) and proportions of individual FAs (g/100 g FAME) in subcutaneous fat of animals fed the five different diets* (n = *6)*

FAs	BDS	WCS	RCS	IRS	MSL	s.e.	Significance†				
							1	2	3	4	5
Total	844	806	858	875	852	48.6	n.s.	n.s.	n.s.	n.s.	n.s.
C12:0	0.772	0.443	0.413	0.197	0.361	0.102	n.s.	*	***	T	n.s.
C14:0	6.38	4.73	4.48	3.77	4.00	0.504	n.s.	**	*	n.s.	n.s.
C16:0	24.4	26.8	23.6	26.3	22.5	1.11	*	n.s.	n.s.	n.s.	T
C18:0	20.8	21.1	21.9	22.2	21.9	1.74	n.s.	n.s.	n.s.	n.s.	n.s.
C18:1 t9	0.198	0.176	0.238	0.169	0.295	0.029	**	n.s.	n.s.	n.s.	n.s.
C18:1t10	0.421	0.335	0.368	0.281	0.828	0.137	**	n.s.	n.s.	n.s.	n.s.
C18:1t11	1.29	0.816	0.916	0.630	1.09	0.175	n.s.	T	*	n.s.	n.s.
C18:1t15	0.369	0.422	0.464	0.645	0.398	0.039	T	n.s.	***	***	n.s.
C18:1c9	28.3	28.4	26.7	29.0	30.8	0.984	*	n.s.	n.s.	n.s.	n.s.
C18:1c15	0.068	0.070	0.081	0.082	0.062	0.010	n.s.	n.s.	n.s.	n.s.	n.s.
C18:2 t11c15	0.214	0.127	0.170	0.165	0.150	0.030	n.s.	T	n.s.	n.s.	n.s.
C18:2 n-6	1.39	1.32	1.63	0.955	2.33	0.145	***	n.s.	*	**	n.s.
CLAc9t11	0.665	0.396	0.412	0.225	0.568	0.098	n.s.	*	**	n.s.	n.s.
CLAt10c12	0.043	0.033	0.042	0.005	0.010	0.008	*	n.s.	**	**	n.s.
CLAcc	0.042	0.034	0.041	0.060	0.024	0.007	*	n.s.	T	*	n.s.
CLAtt	0.037	0.033	0.031	0.041	0.030	0.005	n.s.	n.s.	n.s.	n.s.	n.s.
C18:3c9t11c15	0.032	0.019	0.016	0.012	0.011	0.006	n.s.	T	*	n.s.	n.s.
C18:3 n-3	1.04	1.14	1.42	1.22	1.24	0.125	n.s.	n.s.	n.s.	n.s.	n.s.
Total OBCFA‡	6.22	6.40	6.83	5.24	5.30	0.271	**	n.s.	*	***	n.s.
Total SFA‡	55.6	56.5	54.1	55.2	51.4	1.53	*	n.s.	n.s.	n.s.	n.s.
Total MUFA‡	35.3	34.0	32.7	35.5	38.8	1.16	**	n.s.	n.s.	n.s.	n.s.
Total PUFA‡	3.93	3.57	4.22	3.34	4.93	0.323	**	n.s.	n.s.	n.s.	n.s.
n-6/n-3 ratio‡	1.38	1.16	1.22	0.799	1.90	0.092	***	n.s.	***	**	n.s.
P/S ratio‡	0.047	0.047	0.061	0.042	0.074	0.005	***	n.s.	n.s.	T	T

Abbreviations are: FA: fatty acid; FAME: FA methylesters; BDS = botanically diverse silage; WCS = white clover silage; RCS = red clover silage; IRS = intensive English ryegrass silage; MSL = crushed linseed and maize silage; s.e.: standard error; OBFCA: odd- and branched-chain fatty acid; SFA: saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid.

†T = trend ($0.1 < P < 0.05$); * = $0.05 < P < 0.01$; ** = $0.01 < P < 0.001$; *** = $P < 0.001$; n.s. = non-significant; 1 = orthogonal contrast linseed *v.* forages; 2 = orthogonal contrast BDS *v.* clover diets; 3 = orthogonal contrast BDS *v.* IRS; 4 = orthogonal contrast clover diets *v.* IRS; 5 = orthogonal contrast WCS *v.* RCS.

‡Total OBCFA = sum of all odd- and branched-chain fatty acids: C13:0 *iso*; C13:0 *anteiso*; C13:0; C14:0 *iso*; C15:0 *iso*; C15:0 *anteiso*; C15:0; C16:0 *iso*; C17:0 *iso*; C17:0 *anteiso*; C17:0; C17:1 *c*9. Total SFA=sum of saturated fatty acids: C10:0, C12:0, C13:0; C14:0, C15:0, C16:0, C17:0, C18:0 and C20:0.

Total MUFA = sum of monounsaturated fatty acids: C14:1 *c*9, C15:1 *c*9, C16:1 *t*9, C16:1 *c*9, C17:1 *c*9, C18:1 *t*6-*t*8, C18:1 *t*9, C18:1 *t*10, C18:1 *t*11, C18:1 *t*13+*t*14, C18:1 *t*15, C18:1 *t*16, C18:1 *c*9, C18:1 *c*11, C18:1 *c*12, C18:1 *c*13, C18:1 *c*14, C18:1 *c*15. Total PUFA = sum of polyunsaturated fatty acids: C18:2 *c*9*c*12, C18:2 *t*9*t*12, C18:2 *t*11*c*15, C18:2 n-6, C18:3 n-3, CLA *c*9*t*11, CLA *t*10*c*12, CLA cc, CLA tt and C18:3 *c*9*t*11*c*15.

n-6/n-3 ratio = ratio between C18:2 n-6 and C18:3 n-3. P/S ratio = ratio between the sum of C18:2 n-6 and C18:3 n-3, and the sum of C14:0, C16:0 and C18:0.

the C18:0 and C18:3 n-3 proportions. Nevertheless, higher proportions of C18:2 n-6 were found in the SC fat of animals in group MSL and lower proportions in group IRS. The proportion of CLA *c*9*t*11 in the SC fat was significantly higher for animals of the BDS group compared with groups IRS and WCS+RCS. Concerning other CLA isomers, proportions of CLA *t*10*c*12 were highest for animals in groups BDS, WCS and RCS than for animals of IRS and MSL groups, whereas CLAcc proportions were higher in the SC fat for animals of the IRS group compared with the other groups and proportions of CLAtt were similar between groups.

Neither total concentration of FA nor the proportions of C18:3 n-3 in the IM fat differed between groups (Table 6). Similarly to what was found for the rumen contents, lower proportions of C18:2 n-6 and CLA *c*9*t*11 were found in the IM fat of IRS animals, and proportions of CLA *t*10*c*12 were significantly higher for BDS, WCS and RCS animals compared with the IRS and MSL groups. Proportions of CLAcc tended to be higher for animals of IRS group ($P = 0.082$) compared with BDS group and CLAtt proportions did not differ between groups. Proportions of C18:1 *c*9 tended to be higher in the IM fat of animals in the MSL group compared with the other groups ($P = 0.096$) as seen for the rumen contents. Finally, proportions of C20:4 n-6, C20:5 n-3, C22:5 n-3 and C22:6 n-3 in the IM fat did not differ between groups. Nevertheless, in the muscle of BDS animals, higher C20:5 n-3/C18:3 n-3 and C22:5 n-3/C18:3 n-3 indices for desaturation and elongation activity, as calculated by ratios of product to precursor FA, were observed than in the muscle of MSL and IRS animals. In the muscle of animals fed clover diets (WCS and RCS), higher C20:5 n-3/C18:3 n-3 and C22:5 n-3/C18:3 n-3 indices for desaturation and elongation activity were also observed than in the muscle of IRS animals.

https://doi.org/10.1017/S1751731107000316 Published online by Cambridge University Press

Table 6 *Total concentration (mg/g meat) and proportions of individual FAs (g/100 g FAME) in intramuscular fat of animals fed the five different diets (n = 6)*

FAs	BDS	WCS	RCS	IRS	MSL	s.e.	Significance†				
							1	2	3	4	5
Total	17.9	17.6	18.5	23.8	25.4	3.19	n.s.	n.s.	n.s.	n.s.	n.s.
C12:0	0.422	0.403	0.349	0.226	0.273	0.053	n.s.	n.s.	*	*	n.s.
C14:0	3.58	3.39	3.17	2.71	3.00	0.297	n.s.	n.s.	*	n.s.	n.s.
C16:0	21.1	22.6	21.9	23.6	22.6	0.961	n.s.	n.s.	T	n.s.	n.s.
C18:0	15.5	16.8	17.2	17.3	17.5	0.939	n.s.	n.s.	n.s.	n.s.	n.s.
C18:1 *t*9	0.136	0.144	0.192	0.165	0.253	0.030	*	n.s.	n.s.	n.s.	n.s.
C18:1*t*10	0.345	0.380	0.411	0.247	0.445	0.081	n.s.	n.s.	n.s.	n.s.	n.s.
C18:1*t*11	0.567	0.596	0.645	0.353	0.550	0.095	n.s.	n.s.	n.s.	*	n.s.
C18:1*t*15	0.238	0.249	0.281	0.342	0.279	0.021	n.s.	n.s.	**	**	n.s.
C18:1*c*9	28.5	29.7	28.7	31.3	31.9	1.21	T	n.s.	n.s.	n.s.	n.s.
C18:1*c*15	0.048	0.031	0.015	0.047	0.078	0.016	*	n.s.	n.s.	n.s.	n.s.
C18:2 *t*11*c*15	0.109	0.112	0.148	0.118	0.116	0.024	n.s.	n.s.	n.s.	n.s.	n.s.
C18:2 n-6	4.88	4.15	4.29	3.60	4.95	0.426	n.s.	n.s.	*	n.s.	n.s.
CLA*c*9*t*11	0.595	0.544	0.544	0.301	0.532	0.078	n.s.	n.s.	*	*	n.s.
CLA*t*10*c*12	0.038	0.035	0.036	0.019	0.022	0.005	T	n.s.	**	**	n.s.
CLAcc	0.029	0.035	0.035	0.040	0.026	0.004	T	n.s.	T	n.s.	n.s.
CLAtt	0.025	0.035	0.035	0.031	0.030	0.004	n.s.	T	n.s.	n.s.	n.s.
C18:3*c*9*t*11*c*15	0.039	0.038	0.042	0.029	0.039	0.005	n.s.	n.s.	n.s.	T	n.s.
C18:3 n-3	1.53	1.62	1.82	1.62	1.57	0.151	n.s.	n.s.	n.s.	n.s.	n.s.
C20:4 n-6	2.70	1.90	1.78	1.70	1.46	0.429	n.s.	n.s.	n.s.	n.s.	n.s.
C20:5 n-3	1.08	0.867	0.840	0.826	0.655	0.170	n.s.	n.s.	n.s.	n.s.	n.s.
C22:5 n-3	1.31	1.02	1.03	0.941	0.756	0.185	n.s.	n.s.	n.s.	n.s.	n.s.
C22:6 n-3	0.272	0.245	0.252	0.273	0.233	0.035	n.s.	n.s.	n.s.	n.s.	n.s.
Total OBCFA‡	5.90	5.09	4.93	4.45	4.26	0.390	T	T	*	n.s.	n.s.
Total SFA‡	43.9	45.9	45.3	46.1	45.5	1.16	n.s.	n.s.	n.s.	n.s	n.s.
Total MUFA‡	34.0	35.1	34.4	36.4	37.2	1.13	T	n.s.	n.s.	n.s.	n.s.
Total PUFA‡	13.8	11.6	11.9	10.6	11.3	1.37	n.s.	n.s.	n.s.	n.s.	n.s.
n-6/n-3	1.98	1.79b	1.70	1.63	2.16	0.083	***	*	**	n.s.	n.s.
P/S‡	0.167	0.136	0.148	0.122	0.152	0.019	n.s.	n.s.	T	n.s.	n.s.
Indices for elongation and desaturation activity (calculated as ratios of FA)											
C20:4n-6/C18:2n-6	0.518	0.449	0.410	0.469	0.296	0.055	*	n.s.	n.s.	n.s.	n.s.
C20:5n-3/C18:3n-3	0.702	0.525	0.448	0.489	0.416	0.083	n.s.	*	T	n.s.	n.s.
C22:5n-3/C18:3n-3	0.868	0.628	0.557	0.570	0.489	0.102	n.s.	*	*	n.s.	n.s.
C22:6n-3/C18:3n-3	0.178	0.151	0.144	0.166	0.149	0.017	n.s.	n.s.	n.s.	n.s.	n.s.
C22:5n-3/C20:5n-3	1.25	1.21	1.26	1.19	1.20	0.068	n.s.	n.s.	n.s.	n.s.	n.s.
C22:6n-3/C20:5n-3	0.274	0.300	0.331	0.349	0.359	0.029	n.s.	n.s.	n.s.	n.s.	n.s.
C22:6n-3/C22:5n-3	0.221	0.246	0.261	0.293	0.308	0.021	*	n.s.	n.s.	n.s.	n.s.

Abbreviations are: FA: fatty acid; FAME: FA methylesters; BDS = botanically diverse silage; WCS = white clover silage; RCS = red clover silage; IRS = intensive English ryegrass silage; MSL = crushed linseed and maize silage; s.e.: standard error; OBFCA: odd- and branched-chain fatty acid; SFA: saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid.

†T = trend ($0.1 < P < 0.05$); * = $0.05 < P < 0.01$; ** = $0.01 < P < 0.001$; *** = $P < 0.001$; n.s. = non-significant; 1 = Orthogonal contrast linseed *v.* forages; 2 = orthogonal contrast BDS *v.* clover diets; 3 = orthogonal contrast BDS *v.* IRS; 4 = orthogonal contrast clover diets *v.* IRS; 5 = orthogonal contrast WCS *v.* RCS.

‡Total OBCFA = sum of all odd- and branched-chain fatty acids: C13:0 *iso*; C13:0 *anteiso*; C13:0; C14:0 *iso*; C15:0 *iso*; C15:0 *anteiso*; C15:0; C16:0 *iso*; C17:0 *iso*; C17:0 *anteiso*; C17:0; C17:1 *c*9. Total SFA = sum of saturated fatty acids: C10:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0 and C22:0.

Total MUFA = sum of monounsaturated fatty acids: C14:1 *c*9, C15:1 *c*9, C16:1 *t*9, C16:1 *c*9, C17:1 *c*9, C18:1 *t*6-*t*8, C18:1 *t*9, C18:1 *t*10, C18:1 *t*11, C18:1 *t*13+*t*14, C18:1 *t*15, C18:1 *t*16, C18:1 *c*9, C18:1 *c*11, C18:1 *c*12, C18:1 *c*13, C18:1 *c*14, C18:1 *c*15 and C20:1 *c*9.

Total PUFA = sum of polyunsaturated fatty acids: C18:2 *c*9*c*12, C18:2 *t*9*t*12, C18:2 *t*11*c*15, C18:2 n-6, C18:3 n-6, C18:3 n-3, CLA *c*9*t*11, CLA *t*10*c*12, CLA cc, CLA tt, C18:3 *c*9*t*11*c*15, C20:3 n-6, C20:3 n-3, C20:4 n-6, C20:5 n-3, C22:4 n-6, C22:5 n-3 and C22:6 n-3.

n-6/n-3 ratio = ratio between the sum of C18:2 n-6, C18:3 n-6, C20:3 n-6, C20:4 n-6 and C22:4 n-6, and the sum of C18:3 n-3, C20:3 n-3, C20:5 n-3, C22:5 n-3 and C22:6 n-3. P/S ratio = ratio between the sum of C18:2 n-6 and C18:3 n-3, and the sum of C14:0, C16:0 and C18:0.

Discussion

Rumen fermentation patterns within the four groups fed forage silages were similar. Nevertheless, higher proportions of biohydrogenation intermediates, in particular CLA *c*9*t*11 and C18:1 *t*11 were found in the rumen of BDS animals, despite the similar precursor proportions for the different silages (except for the IRS which presented a higher feed C18:2 n-6+C18:3 n-3 proportion). Microbial markers such as rumen OBCFA (Vlaeminck *et al.*, 2005)

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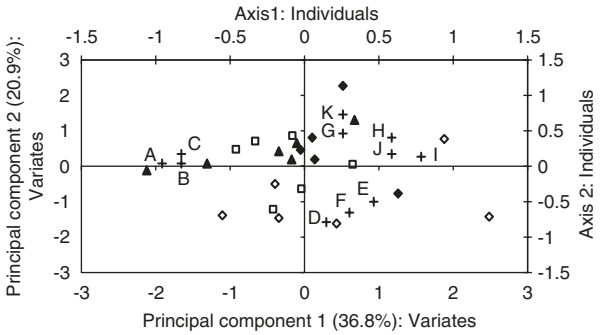

Figure 1 Biplot representing both regression factor scores according to the silage groups (botanically diverse silage (◇), white clover silage (□), red clover silage (▲), intensive ryegrass silage (◆)) and loadings (+) of the first two principal components, based on proportions (% of odd- and branched-chain FA (OBCFA)) of rumen OBCFA. The letters refer to individual OBCFA: A – C15:0; B – *anteiso* C13:0; C – *iso* C14:0; D – *iso* C16:0; E – *iso* C15:0; F – *iso* C13:0; G – C17:1; H – *anteiso* C15:0; I – anteiso C17:0; J – C13:0; K – C17:0.

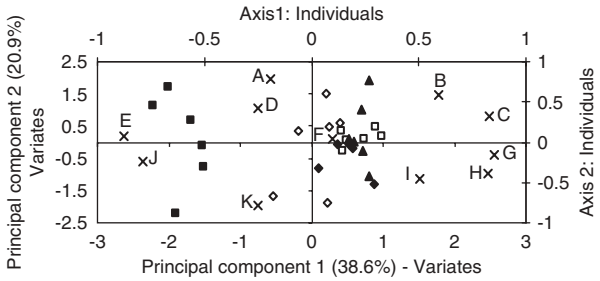

Figure 2 Biplot representing both regression factor scores according to the silage groups (botanically diverse silage (◇), white clover silage (□), red clover silage (▲), intensive ryegrass silage (◆), maize silage and linseed (■)) and loadings (×) of the first two principal components, based on proportions (% of odd- and branched-chain FA (OBCFA)) of rumen OBCFA. The letters refer to individual OBCFA: A – C15:0; B – anteiso C13:0; C – *iso* C14:0; D – *iso* C16:0; E – *iso* C15:0; F – *iso* C13:0; G – C17:1; H – *anteiso* C15:0; I – *anteiso* C17:0; J – C13:0; K – C17:0.

could suggest a different microbial population for the BDS animals. Vlaeminck *et al*. (2006), observed a positive correlation between *iso* C17:0 and C18:1 *t*11, from which they suggested group B bacteria, responsible for the final hydrogenation step, having lower *iso* C17:0 proportions. Further and similar to the results of Lourenço *et al*. (2007), reporting higher rumen *iso* C17:0 proportions in the rumen of BD animals, increased proportions of C16:1 *t*9+*iso* C17:0 were observed in the rumen contents of BDS animals (Table 3). In addition, a PCA analysis (Figure 1) to determine the components which account for most of the variation in OBCFA, revealed a negative score on the second principal component for the BDS animals compared with animals fed the other forage diets (WCS, RCS and IRS), supporting the suggestion of a different microbial population for the BDS animals, based on the proportions of OBCFA observed in the rumen contents of the animals. The suggested different microbial population in the rumen of BDS animals may explain the changes observed in the rumen biohydrogenation intermediates. These suggested differences in microbial population and consequent differences in accumulation of some biohydrogenation intermediates may be due to the presence of compounds in the BDS plant species, which might have antimicrobial activity (Wallace, 2004) and affect rumen fermentation pattern (Busquet *et al*., 2006).

Comparing the forages *v.* linseed feeding, it was clear that rumen contents of MSL animals had the highest C18:1 *t*10 proportions. These higher proportions of C18:1 *t*10 were not associated with a lower rumen pH for the MSL animals, as described by Loor *et al*. (2003 and 2005). Moreover, these animals showed a different fermentation pattern in terms of increased propionate and lower acetate proportions when compared with the other four diets. This pattern was most probably due to the higher starch content of maize (increase of propionate at the expense of acetate, typical for starch-rich concentrate diets (France and Siddons, 1993)). Additionally, the supplementation of PUFA through linseed might also have a methane depressive effect, resulting in a shift of the VFA pattern towards increased propionate proportions (Chilliard *et al*., 2000; Owens *et al*., 2006). Moreover, shifts towards a more amylolytic population could also be suggested from changes in rumen OBCFA, in particular decreases of *iso* C14:0 (Table 3). This FA has also been reported to be negatively correlated with C18:1 *t*10 by Vlaeminck *et al*. (2006), who suggested hydrogenating bacteria responsible for the appearance of C18:1 *t*10 in the rumen to have low proportions of *iso* C14:0. The suggestion for a different microbial population associated with the MSL diet is further illustrated by the PCA biplot (Figure 2), which revealed the lowest first principal component score for the MSL animals compared with the other four groups, with the MSL animals clustering together, based on the proportions of OBCFA observed in the rumen contents of the animals. The different bacterial populations in rumen contents of MSL group could be responsible for the shift of the hydrogenation of C18:2 n-6 from CLA *c*9*t*11 and C18:1 *t*11 to CLA *t*10*c*12 and C18:1 *t*10. Additionally, the higher proportions of C18:1 *t*10 could arise from the isomerisation of C18:1 *t*11 or other C18:1 *trans*-isomers (Proell *et al*., 2002; Loor *et al*., 2005) or from the isomerisation of C18:1 *c*9 (Mosley *et al*., 2002; Loor *et al*., 2005).

Another important finding in this study was the higher C18:3 n-3 proportion in the rumen contents of MSL animals, despite the similar supply of C18 PUFA from the MSL diet compared with the other forage diets (Table 2). This might be due to the presence of C18:3 n-3 in triacylglycerols in crushed linseed whereas the majority of FA in silages are unesterified. Indeed, Lourenço *et al*. (2005a) reported 51% of the silage FA to be in the unesterified form. Additionally, C18:3 n-3 might have been physically protected against microbial attack by the coating of the linseed, which might be effective in impeding the access of the microbial lipases to the C18:3 n-3. Moreover, rumen contents of RCS animals also had significantly higher proportions of C18:3 n-3 compared with the rumen contents of WCS animals. RCSs have been described to increase omega-3 FA in milk of

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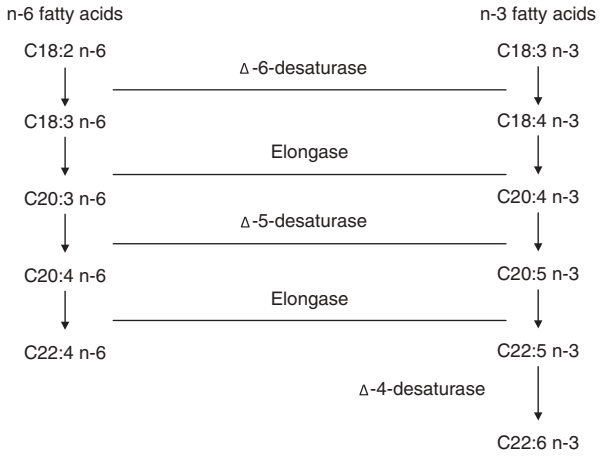

Figure 3 Conversion of C18:2 n-6 and C18:3 n-3 into their long-chain fatty acid products (adapted from Raes *et al.* (2004a)).

cows fed silages (Dewhurst *et al.*, 2003b). This has been hypothesised to be related to higher proportions of esterified FA being protected by the denaturation of plant lipases or to *o*-quinones (produced by polyphenol oxidase (PPO) activity (Jones *et al.*, 1995)) linkages to nucleophilic amino acids of enzymes, e.g. lipases (Lee *et al.*, 2004).

IM fat is known to be less responsive than SC fat to changes in the dietary supply of FA or changes in FA rumen metabolism (Demirel *et al.*, 2004). In this study, FA profile of the SC fat of the animals was a reflection of the rumen data and was more responsive to the changes observed in the rumen FA metabolism than the IM fat. IM fat of BDS animals had the highest, however not significantly different, proportions of most PUFA in line with former studies on pastured lambs (Ådnøy *et al.*, 2005; Lourenço *et al.*, 2007). Although proportions of C20:4 n-6, C20:5 n-3, C22:5 n-3 and C22:6 n-3 did not differ between groups, indices for desaturation and elongation activity did, suggesting that tissue FA metabolism may be influenced by feeding BDSs, similar to the results of Lourenço *et al.* (2007). Moreover, results suggest that the activity of Δ6-desaturase, elongase and Δ5-desaturase (Figure 3) might be affected as BDS animals had higher C20:5 n-3/C18:3 n-3 and C22:5 n-3/C18:3 n-3 indices than the IRS animals, and a higher C20:4 n-6/C18:2 n-6 indices than the WCS and RCS animals. The indices representing the last steps of elongation and desaturation of long-chain FA (Figure 3) in muscle of BDS animals did not differ from WCS, RCS and IRS animals (C22:6 n-3/C18:3 n-3, C22:5 n-3/C20:5 n-3, C22:6 n-3/C20:5 n-3 and C22:6 n-3/C22:5 n-3 indices). This could be due to a negative feed-back of the product FA, limiting this last step of elongation and desaturation of long-chain FA (Raes *et al.*, 2004a). Nevertheless, a confounding effect with the lower IM fat content of these animals and associated higher phospholipid/triacylglycerol ratios and long-chain PUFA proportion cannot be excluded.

This study suggested that feeding different silages induced changes in the rumen FA metabolism which might be related to differences observed in the extent of rumen biohydrogenation of PUFA. The higher rumen C18:3 n-3 concentrations of linseed supplemented animals might be related to its presence in triacylglycerol and a possible physical protection against microbial lipases through the seed coating. Additionally, higher proportions of C18:3 n-3 in the rumen contents of RCS animals were hypothesised to be due to the action of its PPO enzyme. Finally, feeding silages from more botanically diverse pastures could affect tissue FA metabolism as suggested from the indices for desaturation and elongation of PUFA. Overall, these results suggest that animals consuming more BDSs offer opportunities to produce a healthier FA profile from a human health perspective.

Acknowledgements

M. Lourenço acknowledges receipt of a PhD grant from Foundation for Science and Technology – Portugal. Financial support for this experiment is by IWT – Institute for the Promotion of Innovation by Science and Technology in Flanders. Technical assistance of Sabine Coolsaet, Erik Claeys and Stefaan Lescouhier is thankfully acknowledged. We thank Ludo Van Alphen for providing the lambs and the botanically diverse, white clover and red clover silages.

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