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Biohydrogenation and digestion of long chain fatty acids in steers fed on *Lolium perenne* bred for elevated levels of water-soluble carbohydrate

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Abstract — Grass is a rich source of α-linolenic acid (18:3*n*-3) and in this study the effects on ruminal fatty acid metabolism of feeding beef steers zero-grazed *Lolium perenne* containing elevated levels of water-soluble carbohydrate (WSC) were investigated. Eight Hereford × Friesian steers were offered ad libitum access to one of two varieties of *Lolium perenne*, Ba11353, high WSC (HS) or AberElan, intermediate WSC (experimental control) harvested at different times of the day (14:00 and 10:00 h, respectively) to accentuate WSC differentials. The grass was zero-grazed and fed for 21 days, after which the animals were offered ad libitum grass silage for 14 days to provide a covariate intake. The dry matter (202 vs. 167 g per kg fresh weight), WSC (243 vs. 161 g per kg DM), total fatty acids (21.4 vs. 17.9 g per kg DM) and proportion of 18:3*n*-3 (0.54 vs. 0.43) were greater and fibre content was lower (251 vs. 296 g ADF per kg DM) for HS compared with the control. DM intake and intake of total fatty acids and 18:3*n*-3 was higher for HS (9.3 vs. 6.7 kg per d; 201 vs. 117 and 108.5 vs. 51.3 g per d, respectively). There was a trend ($P < 0.1$) for the flow of 18:3*n*-3 at the duodenal to be higher on HS (8.5 vs. 5.7 g per d) but surprisingly there was no significant difference in the flows of 18:0 or 18:1 *trans* (58.5 vs. 48.8 and 11.1 vs. 9.1 g per d, respectively). This may be attributed to the net flows of fatty acids across the rumen (duodenal flow – intake) which were positive on the control and negative on the HS. Biohydrogenation of 18:2*n*-6 and 18:3*n*-3 was not different between treatments and averaged 79.9 and 90.5%, respectively. Intestinal absorption as a proportion of duodenal flow of all the fatty acids were high ranging from 0.70 for 12:0 to 0.96 for 18:1 *trans*. In conclusion, treatment HS a *Lolium perenne* bred for elevated levels of WSC had higher total fatty acids and a higher proportion of the beneficial fatty acid 18:3*n*-3 compared to a control. The higher DM intakes achieved when feeding the treatment HS along with the greater content of 18:3*n*-3 resulted in a trend for greater intakes of this fatty acid and flow to and absorption from the small intestine.

rumen / fatty acids / linolenic acid / grass / digestion

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Résumé — Biohydrogénation et digestion des acides gras chez le bouvillon recevant du ray-grass anglais sélectionné pour une teneur élevée en glucides solubles. L'herbe est une source importante d'acide α-linolénique (18:3*n*-3). Dans cet essai, on a étudié sur des bouvillons les effets sur le métabolisme ruminal des acides gras de la distribution à l'auge de ray-grass anglais sélectionné pour une teneur élevée en glucides solubles (GS). Huit bouvillons Hereford × Frison ont reçu ad libitum une des deux variétés de ray-grass anglais (*Lolium perenne*) Ba11453 à haute teneur en GS (HGS), ou AberElan, à teneur intermédiaire en GS (témoin), récoltées à des heures différentes (14 et 10 h respectivement) pour accroître la différence de concentration en GS. L'herbe était coupée et distribuée à l'auge pendant 21 jours, après quoi les animaux ont reçu de l'ensilage d'herbe ad libitum pendant 14 jours afin de fournir une valeur d'ingestion utilisée comme covariable. Les teneurs en matière sèche (MS) (202 vs. 167 g par kg frais), en GS (243 vs. 161 g par kg MS), en acides gras totaux (21,4 vs. 17,9 g par kg MS), et la proportion de 18:3*n*-3 dans les acides gras (0,54 vs. 0,43) étaient plus élevées, et la teneur en parois (251 vs. 296 g ADF par kg MS) plus faible pour HGS que pour le témoin. Les ingestions journalières de MS, d'acides gras et de 18:3*n*-3 ont été plus élevées pour HGS (9,3 vs. 6,7 kg, 201 vs. 117 g et 108,5 vs. 51,3 g, respectivement). Il y a eu une tendance ($P < 0.1$) à un accroissement du flux duodénal de 18:3*n*-3 pour HGS (8,5 vs. 5,7 g par j) mais curieusement il n'y a pas eu de différence de flux de 18:0 ou 18:1 *trans* (58,5 vs. 48,8 et 11,1 vs. 9,1 g par j, respectivement). Cela pourrait être attribué au fait que les flux nets d'acides gras (flux duodénal – ingéré) ont été positifs pour HGS et négatifs pour le témoin. La biohydrogénation du 18:2*n*-6 et du 18:3*n*-3 n'a pas varié avec le traitement et a été respectivement de 79,9 et 90,5 %. L'absorption intestinale, en proportion du flux duodénal d'acides gras, a été élevée, allant de 0,70 pour le 12:0 à 0,96 pour le 18:1 *trans*. En conclusion, le traitement HGS contenait plus d'acides gras totaux et plus d'acides gras à effets bénéfiques sur la santé humaine que le témoin. L'ingestion plus élevée de MS avec HGS a entraîné une tendance à une ingestion, un flux duodénal et une absorption intestinale plus élevées de 18:3*n*-3.

rumen / acides gras / acide linolénique / herbe / digestion

1. INTRODUCTION

Meat and meat products are an important part of today's diet and make an important contribution to nutritional intakes [2]. However, the fatty acid composition of ruminant products has become increasingly important in recent years because even though they may be low in fat, the neutral marbling fat is relatively saturated. For example in beef approximately 47, 42 and 4% of total fatty acids are saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), respectively [19]. Current research is oriented towards reducing the amounts of SFA and increasing beneficial PUFA in particular α-linolenic acid (18:3*n*-3) and the longer chain C_{20} PUFA eicosapentaenoic acid (20:5*n*-3) and docosahexaenoic acid (22:6*n*-3) [2–4, 28].

Increasing the dietary supply of *n*-3 PUFA is one of the most important strategies to increase the PUFA composition in the product (milk or meat). Hence, feeding linseed as a rich source of 18:3*n*-3 increases the levels of this fatty acid in the meat and also its longer chain derivative 20:5*n*-3 [3, 28]. Grass is also rich in 18:3*n*-3 and offers considerable potential in many European areas as a natural home grown forage to improve the fatty acid composition of ruminant products. However, one of the limitations to manipulating the fatty acid composition of ruminant products is the fact that glycerol based dietary lipids are extensively hydrolysed by rumen micro-organisms, leading to the formation of free fatty acids [21]. It is known that this lipolysis is rapid but may be decreased by antibiotics and a low pH [4]. Once the ester bond has been cleaved, free unsaturated fatty acids are rapidly hydrogenated by micro-organisms to more saturated end products, in the process of biohydrogenation, this results in an increase in the SFA concentration

of the ruminant product [24]. The extent to which biohydrogenation occurs is variable and in an extensive review of the literature Doreau and Ferlay [6] found that biohydrogenation of 18:2*n*-6 and 18:3*n*-3 ranged between 70–95% and 85–100% (average 80 and 92%), respectively. Linoleic is often incompletely biohydrogenated resulting in different mono-unsaturated fatty acids, of which vaccenic acid (*trans*-11 18:1) is most prominent, in addition to stearic acid. It also results in the formation of conjugated linoleic acids (CLA) of which *cis*-9, *trans*-11 is the most dominant. The production of *cis*-9, *trans*-11 CLA is increased as 18:2*n*-6 intake increases, suggesting that the capacity of microbes to hydrogenate may be overcome by high levels of unsaturated fatty acids [16].

The major glycerol based lipid classes in grasses are phospho- and galacto-diacylglycerols, as opposed to the triacylglycerols of many oil seeds (e.g. linseed). The literature contains relatively little information on the biohydrogenation of these lipid classes contained in grass [1, 15, 23], compared to the many studies focused on oil seeds [10, 16, 22, 29]. Recent studies at our Institute have examined the effects of feeding *Lolium perenne* selected for elevated levels of water-soluble carbohydrate (WSC) on animal performance and efficiency of dietary nitrogen utilisation [14, 18]. A further study was conducted to assess the effects on ruminal fatty acid metabolism of feeding beef steers zero-grazed *Lolium perenne* with different WSC concentrations. The effects on ruminal fatty acid metabolism are reported in this paper. It was speculated that feeding grass with a higher WSC content might reduce ruminal biohydrogenation of dietary PUFA as a result of a lower ruminal pH in the same manner that feeding higher levels of concentrates may reduce biohydrogenation as a result of pH changes in the rumen [4]. The effects on other aspects of rumen function and amino acid absorption were reported by Lee et al. [13].

2. MATERIALS AND METHODS

2.1. Experimental design

The experimental design was described previously [13]. In brief, eight Hereford × Friesian steers, initial live weight 430 (s.e. 9.0) kg, prepared with a rumen cannula and simple 'T'-piece cannulae in the proximal duodenum and terminal ileum were individually offered at 09:00 and 16:0 h ad libitum access to zero-grazed *Lolium perenne*. Refusals were collected at 08:45 and 15:45 h to determine daily DM intake. The experiment took place between April and May 2000 and hence the grass used was of primary growth with the aim of using material before it reached 50% ear emergence. The diets were a high water-soluble carbohydrate (WSC) variety (HS; var. Ba11353) harvested at approximately 14:00 h and a control variety (AberElan) harvested at approximately 10:00 h. The grasses were harvested at different times to accentuate differences in WSC concentration. The experiment consisted of one main 21 day experimental period, 14 days for adaptation to the diet and 7 days for collection of rumen, duodenal and ileal digesta. Faecal collections were not made and therefore measurements of whole tract digestibility of measured components are not available. Following the end of the experimental period, animals were gradually changed onto a standard experimental silage for a 14 day period after which daily feed intakes were monitored for a further 5 days as a covariate measurement. The single period design with a covariate measurement on silage was chosen to minimise the effects of the large seasonal variations observed in grass chemical composition [25].

2.2. Sampling

Digesta flow at the duodenum and ileum were estimated using a dual-phase marker system with ytterbium acetate (YbAc) and

chromium ethylene diamine tetra-acetic acid (CrEDTA) as the particulate and liquid markers, respectively [9]. The markers (YbAc: 50 mg Yb per kg DM intake; CrEDTA: 3700 mg per d) were infused continuously by a peristaltic pump at a rate of 30 mL per h for 6 days prior to digesta sampling. On day 15 of the experiment ileal digesta was collected manually every 4 h over a 24 h period. This was followed by a rest day before duodenal digesta was collected for two consecutive days (day 17–18) in the same manner. Diet changeovers commenced for the covariate period after completion of sampling.

2.3. Sample preparation and analysis

Sub-samples of digesta were either stored frozen (–20 °C) or freeze-dried, ground and retained for chemical analysis. Accumulated samples of daily duodenal and ileal digesta were thoroughly mixed and a 200 g sub-sample freeze-dried representing whole digesta. A separate 200 g portion was centrifuged at 3000 × *g* for 25 min to provide the centrifuged solid digesta. These were subsequently freeze-dried, ground and retained frozen for analysis. Grass samples were collected twice daily prior to feeding and bulked for each day of the digesta collection period, they were then freeze-dried, ground and stored frozen (–20 °C) prior to fatty acid analysis.

Chemical compositions of the grasses were determined as described by Lee et al. [13]. Grass fatty acids were obtained by direct hydrolysis, with added internal standard (heneicosanic acid methyl ester; Sigma, Poole, Dorset, UK), in 5 M potassium hydroxide in aqueous methanol followed by acid hydrolysis at pH 0.5 after adjustment with 10 N sulphuric acid. Digesta fatty acids were prepared similarly but omitting the final acid hydrolysis. Fatty acids were converted to methyl esters with diazomethane and analysed by gas liquid chromatography on a CP Sil 88 FAME column (50 m × 0.25 mm ID, Chrompack, UK Ltd., London) with split injection [8]. Peaks were identified from external standards (ME61, Larodan fine chemicals, Malmo, Sweden; S37, Supelco, Poole, Dorset, UK; CLAs, Matreya, Philadelphia, USA) and quantified using the internal standard. Results are reported for major fatty acids and those PUFA of relevance to the study. The *trans* isomers of 18:1 were not fully resolved and are reported as a single value.

2.4. Calculations and statistical analysis

Digesta flows were calculated after mathematical reconstitution of true digesta as described by Faichney [9]. Digesta flow parameters and intake data were averaged across days, blocked according to steer and subjected to a general analysis of variance (HS vs. control) using DM intake (measured during the covariate period) as a covariate (Genstat 5 statistical package [12]), where significance was defined as $P = 0.05$. Individual sample data of the chemical compositions of the feeds were subjected to a general analysis of variance using Genstat 5 statistical package [12].

3. RESULTS

The chemical composition of the herbage is presented in Table I. WSC content was approximately 83 g per kg DM higher while NDF and ADF were approximately 83 and 44 g per kg DM lower in HS compared to the control. This contributed towards a higher in vitro dry matter digestibility for HS. No differences in total nitrogen were found. The fatty acid composition of the herbage is given in Table II. Total fatty acids were higher in HS. Higher levels of 16:0, 18:0 and 18:3*n*-3 were found in the HS while levels of 18:1*n*-9 were lower compared to the control. The proportions of the principle fatty acids were different with

Table I. Chemical composition (g per kg DM unless otherwise stated) of the two grass diets Ba11353 high WSC (HS) and the control (AberElan; g per kg DM) (residual degrees of freedom = 11) (Lee et al. [13]].

	Control	HS	s.e.d.	*P*
Dry matter (g DM per kg fresh)	167.0	202.0	1.23	0.005
Organic matter	942.9	936.1	2.01	0.005
Water soluble carbohydrate	160.7	243.2	8.72	0.001
Total nitrogen	15.9	16.6	0.66	NS
Acid detergent fibre	295.7	251.4	7.16	0.001
Neutral detergent fibre	562.5	479.6	12.24	0.001
IVDMD	0.56	0.61	0.014	0.007

s.e.d.: standard error of the difference; *P*: significance effects between the two diets, NS symbolises not significant at $P < 0.05$; IVDMD: in vitro dry matter digestibility.

Table II. Fatty acid composition (g per kg DM) of the two grass diets Ba11353 high WSC (HS) and the control AberElan (residual degrees of freedom = 11).

	Control	HS	s.e.d.	*P*
12:0 myristic	0.10	0.08	0.007	NS
14:0 lauric	0.36	0.31	0.037	NS
16:0 palmitic	2.80	3.01	0.033	0.001
16:1 palmitoleic	0.25	0.31	0.008	0.001
18:0 stearic	0.29	0.32	0.007	0.001
18:1*n-9* oleic	0.57	0.45	0.009	0.001
18:2*n-6* linoleic	3.14	3.09	0.042	NS
18:3*n-3* linolenic	7.80	11.50	0.235	0.001
		Proportion of total fatty acids		
16:0 palmitic	0.17	0.14	0.001	0.001
18:2*n-6* linoleic	0.18	0.14	0.001	0.001
18:3*n-3* linolenic	0.43	0.54	0.005	0.001
Total fatty acids	17.9	21.4	0.318	0.001

s.e.d.: standard error of the difference.

16:0 and 18:2*n*-6 lower and 18:3*n*-3 higher on HS relative to the control.

Intake and duodenal flow of fatty acids are presented in Table III. The increased DM intake on HS and the higher content of total fatty acids in this grass resulted in a substantially higher intake (84 g per d) of total fatty acids. As expected the major intake of fatty acids were as 16:0, 18:2*n*-6 and 18:3*n*-3 representing approximately 14, 14 and 54 and 16, 17 and 44% on the HS and control, respectively.

Total fatty acid flow to the duodenum was not significantly different between the treatments. In comparison with intake, flow of 12:0, 14:0, 16:0, 18:0 and 18:1 *trans* to the duodenum all increased while that of the unsaturated fatty acids 18:2*n*-6 and 18:3*n*-3, decreased. Flow of 18:3*n*-3 at the duodenum tended to be higher on HS

Table III. Dry matter and fatty acid intake and flow to the duodenum in steers fed the two grass diets Ba11353 high WSC (HS) and control (AberElan) (residual degrees of freedom = 6).

	Control	HS	s.e.d.	*P*
Dry matter				
Intake (kg per d)	6.66	9.27	0.179	0.001
Duodenal flow (kg per d)	3.06	3.82	0.302	0.05
Fatty acid intake (g per d)				
12:0 myristic	0.60	0.78	0.018	0.001
14:0 lauric	2.2	2.9	0.07	0.001
16:0 palmitic	18.3	28.2	0.56	0.001
16:1 palmitoleic	1.6	2.9	0.05	0.001
18:0 stearic	1.9	3.0	0.06	0.001
18:1*n-9* oleic	3.7	4.2	0.11	0.005
18:1*trans*	ND	ND		
18:2*n-6* linoleic	20.3	29.2	0.62	0.001
18:3*n-3* linolenic	51.3	108.5	1.62	0.001
Total fatty acids	117	201.1	3.59	0.001
Fatty acid flow to duodenum (g per d)				
12:0 myristic	2.0	2.5	0.95	NS
14:0 lauric	4.5	5.8	1.08	NS
16:0 palmitic	21.3	22.9	3.43	NS
16:1 palmitoleic	6.1	5.6	1.41	NS
18:0 stearic	48.8	58.5	7.47	NS
18:1*n-9* oleic	3.4	4.4	0.83	NS
18:1*trans*	9.1	11.1	4.41	NS
18:2*n-6* linoleic	4.5	5.4	1.16	NS
18:3*n-3* linolenic	5.7	8.5	1.75	0.09
Total fatty acids	137.9	165.8	20.66	NS

s.e.d.: standard error of the different; ND: not detected.

compared to the control. No CLA (*cis*-9, *trans*-11 CLA) was detected in duodenal digesta, when the traces were compared against the external standards. Net flow (calculated as intake minus duodenal flow) of fatty acids is given in Table IV. A negative net flow suggests disappearance of that fatty acid from the rumen via biohydrogenation, absorption or metabolism. A positive net flow of fatty acid indicates net appearance or synthesis in the rumen from biohydrogenation, de novo fatty acid synthesis by ruminal micro-organisms or from endogenous lipid. Interestingly, there was a net loss of total fatty acids with HS relative to net gain with the control (–35.4 vs. 20.9 g per d, respectively). A net gain of 16:0 was noted on the control relative to a loss with the HS. The net loss of 18:2*n*-6 and 18:3*n*-3 across the rumen was greater on HS compared to control and since duodenal flows of these PUFA were similar,

Table IV. Net flow of fatty acids at the duodenum (g per d; duodenal flow minus intake) in steers fed the two grass diets Ba11353 high WSC (HS) and control (AberElan) (residual degrees of freedom = 6).

	Control	HS	s.e.d.	*P*
12:0 myristic	1.4	1.7	0.93	NS
14:0 lauric	2.3	2.9	1.03	NS
16:0 palmitic	3.0	–5.3	3.17	0.045
16:1 palmitoleic	4.5	2.7	1.39	NS
18:0 stearic	46.9	55.5	7.46	NS
18:1*n-9* oleic	–0.3	0.2	0.78	NS
18:1*trans*	9.1	11.1	4.41	NS
18:2*n-6* linoleic	–15.8	–23.8	0.65	0.001
18:3*n-3* linolenic	–45.6	–100.0	0.51	0.001
Total fatty acids	20.9	–35.3	17.91	0.026

s.e.d.: standard error of the difference.

Table V. Biohydrogenation (%) of unsaturated fatty acids in the rumen of steers fed on the two grass diets Ba11353 high WSC (HS) and control (AberElan) (residual degrees of freedom = 6).

	Control	HS	s.e.d.	*P*
18:2*n-6* linoleic	78.3	81.5	4.45	NS
18:3*n-3* linolenic	89.2	91.8	2.48	NS

s.e.d.: standard error of the difference.

Table VI. Absorption of fatty acid from the small intestine of steers fed on the two grass diets Ba11353 high WSC (HS) and the control AberElan (residual degrees of freedom = 6).

	Control	HS	s.e.d.	*P*
	(as a proportion of duodenal flow)			
12:0 myristic	0.728	0.699	0.0772	NS
14:0 lauric	0.851	0.828	0.0417	NS
16:0 palmitic	0.871	0.851	0.0248	NS
16:1 palmitoleic	0.889	0.829	0.0381	NS
18:0 stearic	0.891	0.861	0.0311	NS
18:1*n-9* oleic	0.833	0.848	0.0481	NS
18:1*trans*	0.961	0.951	0.0114	NS
18:2*n-6* linoleic	0.875	0.901	0.0147	NS
18:3*n-3* linolenic	0.911	0.922	0.0088	NS
Total fatty acids	0.931	0.946	0.0105	NS

s.e.d.: standard error of the difference.

this difference in net flow reflects differences in intake of these fatty acids.

Biohydrogenation of the PUFA, 18:2*n*-6 and 18:3*n*-3 is given in Table V. Biohydrogenation of both these fatty acids was high and not different between treatments averaging 79.9 and 90.5% for 18:2*n*-6 and 18:3*n*-3, respectively. Small intestinal absorption of fatty acids is presented in Table VI. This was calculated as the net difference between duodenal and ileal fatty acid flow as a function of fatty acid duodenal flow. The absorption coefficients of all fatty acids were high and no significant differences were found between treatments.

4. DISCUSSION

The HS grass treatment did have a higher content of WSC and lower NDF and ADF fractions. The grasses were cut at different times of the day to ensure an enhanced WSC differential between the treatments. As discussed by Lee et al. [13] these differences are likely to be the combined effect of genotypic and environmental/diurnal factors. The difference in time of harvesting the two grasses also resulted in the HS grass having a greater DM content (35 g DM per kg fresh weight). Total grass fatty acids were in the range typically quoted for grass [5, 17] but interestingly, the HS grass had a higher lipid content (21.4 vs. 17.9 g per kg DM for HS vs. control, respectively) and a higher proportion of α-linolenic acid. The concentration of fatty acids in herbage tends to be highest in Spring and Autumn, with lowest values during Summer, particularly around flowering (note the material used in this study was harvested in early-mid May, with grass heading dates of 29th May and 2nd June for the control and HS, respectively). This effect has been noted for perennial ryegrass (*Lolium perenne*) by Bauchart et al. [1] and for cocksfoot (*Dactylis glomerata*) and white clover (*Trifolium repens latum*) by Saito et al. [26]. Dewhurst et al. [5] showed a more pronounced decline in fatty acid content for hybrid ryegrass and, particularly, for Italian ryegrass (*Lolium multiflorum*) around flowering. It has also been demonstrated that fatty acid profiles were distinctive to individual grass species when the grasses received the same management (i.e. at the same cut), confirming a strong genetic basis. Recent studies at our Institute have demonstrated that in a well-characterised population of perennial ryegrass, a strong positive relationship existed between total fatty acids and the proportion of 18:3*n*-3 in the grass. Preliminary evidence for significant Quantitative Trait Loci for concentrations of several of the important fatty acids in this population were also noted (LB Turner, personal communication).

The reasons for the increase in DM intake on the HS treatment compared to the control were discussed in depth by Lee et al. [13] and are thought to relate to (1) increased WSC and reduced fibre concentration, (2) reductions in rumen ammonia effecting satiety and (3) increase in DM content. Differences in net flow of total fatty acids were found between the treatments. Relative to intake, flow was increased on the control treatment by 20.9 g per d (17.9% increase) but was reduced on the HS treatment by 35.4 g per d (17.6% reduction). Negative flows of fatty acids are sometimes observed when supplementing with lipids [6, 16, 34] or with fresh forages [23, 30] suggesting losses due to absorption across the rumen wall and/or microbial degradation to other compounds. Others, however, have reported positive flows in response to feeding lipids to steers [29] or sheep [33]. Methodological problems associated with measuring duodenal dry matter flow are sometimes implicated as a potential reason for disappearance of fatty acids in the rumen [20, 34] although there is little to suggest that this may explain part of the loss in this study. Few studies have been published which have examined ruminal

fatty acid metabolism of fresh forages. Bauchart et al. [1] examined the effects of structural and chemical changes in fresh ryegrass, given as the only feed from Spring to Autumn (7 measurement periods) on fatty acid metabolism in the rumen and apparent intestinal absorption. In all periods examined, fatty acid flows into the duodenum were less than corresponding dietary intakes (–15.7 to –32.6% of intake) except in the stemmy regrowth where positive flows were noted (+11.2–20.2%). Similar observations were noted by Outen et al. [23] in sheep fed red clover and suggestions of negative fatty acid balances across the rumen in sheep fed fresh forage were reported by Ulyatt and MacRae [30]. In studies with silage Lee et al. [15] found positive flows for grass and mixtures of grass and clover but negative flows for pure clover diets. The higher duodenal flows of 18:0 compared with intake on both treatments reflect ruminal biohydrogenation of unsaturated fatty acids. Similarly 18:1 *trans* isomers, of which vaccenic acid is the most prominent, is a product of biohydrogenation and hence duodenal flows are higher than intake. Data on ruminal metabolism of forage lipids has shown extensive biohydrogenation (c. 85% for 18:2*n-6* and c. 90% for 18:3*n-3*) of long chain PUFA on these diets with reference to fresh forage in this study and conserved forage in the study of Lee et al. [15]. Doreau and Poncet [7] also noted high levels of biohydrogenation of 18:2*n*-6 and 18:3*n*-3 with forage diets but for 18:3*n*-3 they observed lower levels of biohydrogenation in hay compared with fresh forage. As a consequence of this extensive biohydrogenation there are low duodenal flows of its by-products, namely *cis* 9 *trans* 11 CLA, and 18:1 *trans*. In this study *cis* 9 *trans* 11 CLA was not detected and in the study of Lee et al. [15] they were low, in the region of 0.7 g per d. In some situations 18:1 *trans* isomers accumulates and high duodenal flows of this fatty acid may be observed. This is thought to be a result of an inhibition of terminal biohydrogenation of 18:1 *trans* to 18:0 when supplementing with a lipid rich diet [29, 33]. However, in this study duodenal flow of 18:1 *trans* averaged 10.1 g per d compared with approximately 100 g per d in steers with similar total DM intakes but receiving 40% of their total DM intake as a concentrate containing either linseed or fish oil [29]. Duodenal flows of 18:1 *trans* isomers in this study are in the same range noted by Lee et al. [15]. In studies using sheep, Kucuk et al. [11] and Sasaki et al. [27] noted an increase in 18:1 *trans* (vaccenic acid) and a reduction in biohydrogenation with increasing amounts of concentrate and decreasing amounts of forage in the diet. They also found a greater flow of *cis* 9 *trans* 11 CLA with increasing forage in the diet.

The extent of biohydrogenation is a critical factor in determining the degree of success in being able to modify the fatty acid composition of ruminant products. In this study biohydrogenation of 18:2*n*-6 and 18:3*n*-3 was high and not different between treatments, averaging approximately 80 and 91%, respectively. It was originally speculated that feeding grass with a higher content of WSC might influence ruminal pH and consequently influence lipolysis and/or biohydrogenation. Hence, the lack of difference between the treatments is not surprising since the difference in ruminal pH was numerically very small between the treatments, averaging 6.3 vs. 6.4 ($P < 0.01$) for the control and HS, respectively. Van Nevel and Demeyer [31, 32] showed that in in vitro systems a reduction in pH below 6 resulted in a 20% reduction in lipolysis and a smaller reduction in biohydrogenation (5%). Hence, with a similar extent of biohydrogenation between treatments, the HS grass tended to have higher duodenal flow of 18:3*n*-3 (8.5 vs. 5.7 g per d for HS and control, respectively), reflecting the greater intake of 18:3*n*-3 on this diet. These flows are comparable with those in steers fed on a concentrate containing linseed diet, a rich source of 18:3*n-3,* where flows

of 10 g per d were measured [29]. In this study, intake of oleic acid (18:1*n*-9) was very low reflecting the low proportion of this monounsaturated fatty acid in grass (Tab. III) and consequently duodenal flows were also low and the error around the mean values was high. Therefore, it was not meaningful to calculate biohydrogenation values for this fatty acid, which is normally lower than those for 18:2*n*-6 and 18:3*n*-3 (typically 50–70%) [10, 29].

Small intestinal digestibility and hence the amount of fatty acid which is available for absorption is also a major step in manipulating the fatty acid composition of ruminant products. Digestibility of all the C_{18} fatty acids was high ranging from 0.83–0.92 for 18:0, 18:1*n*-9, 18:2*n*-6 and 18:3*n*-3, with no differences between treatments. In general, fatty acid digestibility depends on chain length and degree of saturation. Doreau and Ferlay [6] calculated that the average digestibility of C_{18} fatty acids is 0.77, 0.85, 0.83 and 0.76 for 0, 1, 2 and 3 double bonds, respectively, as unsaturated fatty acids are more hydrophilic and therefore form micelles more readily and are thus readily absorbed. In this study, 18:3*n*-3 is particularly important as the major fatty acid in grass and since digestibility of 18:3*n*-3 was not different between treatments then, as expected, the greatest amount of 18:3*n*-3 absorption occurred on the HS treatment.

5. CONCLUSIONS

In conclusion, treatment HS, a *Lolium perenne* variety bred for elevated levels of WSC, had higher total fatty acids and proportion of the beneficial fatty acid 18:3*n*-3 compared to a control. The higher DM intakes achieved when feeding the HS variety along with the greater content of 18:3*n*-3 resulted in greater intakes of this fatty acid and a trend towards a greater flow to and absorption from the small intestine. However, biohydrogenation of C_{18} PUFA were not different between treatments.

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