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Original Research Article

Sainfoin (*Onobrychis viciifolia*) silage in dairy cow rations reduces ruminal biohydrogenation and increases transfer efficiencies of unsaturated fatty acids from feed to milk

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ABSTRACT

The effects of replacing grass silage by sainfoin silage in a total mixed ration (TMR) based diet on fatty acid (FA) reticular inflow and milk FA profile of dairy cows was investigated. The experiment followed a crossover design with 2 dietary treatments. The control diet consisted of grass silage, corn silage, concentrate and linseed. In the sainfoin diet, half of the grass silage was replaced by a sainfoin silage. Six rumen cannulated lactating multiparous dairy cows with a metabolic body weight of 132.5 ± 3.6 kg $BW^{0.75}$, 214 ± 72 d in milk and an average milk production of 23.1 ± 2.8 kg/d were used in the experiment. Cows were paired based on parity and milk production. Within pairs, cows were randomly assigned to either the control diet or the sainfoin diet for 2 experimental periods (29 d per period). In each period, the first 21 d, cows were housed individually in tie-stalls for adaptation, then next 4 d cows were housed individually in climate-controlled respiration chambers to measure CH_4. During the last 4 d, cows were housed individually in tie stalls to measure milk FA profile and determine FA reticular inflow using the reticular sampling technique with Cr-ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) and Yb-acetate used as digesta flow markers. Although the dietary C18:3n-3 intake was lower ($P = 0.025$) in the sainfoin diet group, the mono-unsaturated FA reticular inflow was greater ($P = 0.042$) in cows fed the sainfoin diet. The reticular inflow of trans-9, trans-12-C18:2 and cis-12, trans-10 C18:2 was greater ($P \leq 0.024$) in the sainfoin diet group. The cows fed sainfoin diet had a lower ($P \leq 0.038$) apparent ruminal biohydrogenation of cis-9-C18:1 and C18:3n-3, compared to the cows fed the control diet. The sainfoin diet group had greater ($P \leq 0.018$) C18:3n-3 and cis-9, cis-12-C18:2 proportions in the milk FA profile compared to the control diet group. Transfer efficiencies from feed to milk of C18:2, C18:3n-3 and unsaturated FA were greater ($P \leq 0.0179$) for the sainfoin diet. Based on the results, it could be concluded that replacing grass silage by sainfoin silage in dairy cow rations reduces ruminal C18:3n-3 biohydrogenation and improves milk FA profile.

1. Introduction

Roughages, especially fresh and ensiled grass are mainly feed for ruminants. These roughages have a high linolenic acid (cis-9, cis-12, cis-15-C18:3 or C18:3n-3) content (Elgersma et al., 2003). However, apparent transfer efficiency of C18:3n-3 from ingested feed into milk is very low (Glasser et al., 2008) due to extensive biohydrogenation of C18:3n-3 or their intermediates by ruminal bacteria (Harfoot et al., 1997).

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Lactating cows have been fed diets with various vegetable oils or oilseeds to improve the transfer efficiencies of mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA) from dietary fat to milk fat (Loor et al., 2005; Shingfield et al., 2008; Sterk et al., 2012a). In addition, some studies showed that condensed tannins (CT) from various legume forages inhibited the growth of many ruminal bacteria, including bacteria associated with ruminal biohydrogenation (Jones et al., 1994; Vasta et al., 2010). CT from *Dorycnium rectum* forage inhibited the growth of *Butyrivibrio fibrisolvens* bacteria which are involved in the ruminal biohydrogenation process (Sivakumaran et al., 2004; Vasta et al., 2010). Supplementation of CT (78.9 g/kg DM) in rumen simulation technique (RUSITEC) inhibited the last step of C18:3n-3 biohydrogenation (Khiaosa-Ard et al., 2009). Feeding quebracho tannins in the diet of sheep resulted in an increased concentration of trans-11-C18:1 in the rumen (Vasta et al., 2009a, 2010) and increased concentrations of cis-9, trans-11-C18:2 and PUFA in lamb meat (Vasta et al., 2009b). Moreover, supplementation with quebracho tannin extract at 30 g/kg of DM diet, increased the content of C18:3n-3 in milk, compared to a control diet (Dschaak et al., 2011).

Sainfoin (*Onobrychis viciifolia*) is a tanniniferous legume that is grown in dry hilly environments on calcareous soils of Europe, Asia, and the western part of North America. Because of its high protein content and palatability (Scharenberg et al., 2007), it is a useful fodder for grazing animals or when fed as a hay or silage (Hayot Carbonero et al., 2011). Feeding sainfoin for ruminants prevented bloat (McMahon et al., 1999) and reduced the parasitic load (Hoste et al., 2015). Moreover, sainfoin has been shown to reduce enteric CH_4 emission from dairy cows in vitro (Hatew et al., 2016) and in vivo (Huyen et al., 2016). Ruminants fed sainfoin had a lower protein degradation level in the rumen, compared to those fed lucerne (Kraiem et al., 1990; Aufrère et al., 2013). Sainfoin pellets increased milk unsaturated fatty acids (UFA) in lactating cows (Girard et al., 2015) due to CT modulating the activity of bacteria involved in biohydogenation processes (Vasta et al., 2010). Based on database of scientific publications, the authors found that this is the first study with sainfoin on the combination of quantifying between FA reticular inflow and biohydrogenation and MUFA or PUFA transfer into milk. The objective of this study was to determine the extent of rumen biohydrogenation of C18:3n-3 and FA composition in milk when lactating cows were fed a sainfoin silage (a CT-containing forage) compared to grass silage alone (a CT-free forage) in total mixed ration (TMR). The hypothesis of the current study was that replacing grass silage by sainfoin silage in dairy cow rations would reduce ruminal biohydrogenation and increase PUFA in milk.

2. Materials and methods

2.1. Experimental design, animals and housing

The experiment was approved by the Institutional Animal Care and Use Committee of Wageningen University under the Dutch Law on Animal Experimentation. The experiment followed a crossover design with 2 dietary treatments. A total of 6 rumen cannulated (Type 1C; Bar Diamond Inc., Parma, ID, USA) lactating multiparous Holstein Friesian dairy cows with a metabolic body weight of 132.5 ± 3.6 kg $BW^{0.75}$ (mean ± SD), 214 ± 72 d in milk and an average milk production of 23.1 ± 2.8 kg/d were used in the experiment. Cows were paired based on parity and milk production. Within pairs, cows were randomly assigned to receive either a control diet or a sainfoin diet (SAIN group) (Table 1) for an experimental period of 29 d whereafter cows were switched for dietary treatment and followed for another 29-d experimental period. Prior to each experimental period, all cows received the control diet for 7 d before receiving the dietary treatment.

During the first 21 d of each 29-d experimental period, cows were housed in tie stalls for adaptation. From d 22 to 25 of experimental period, cows were housed individually in climate-controlled respiration chambers (CRC) to measure CH_4 production, apparent total tract digestibility, energy and nitrogen (N) balance and milk production (data reported by Huyen et al., 2016). Then, cows were returned to the tie stalls for 4 d (d 26 to 29) to determine the extent of biohydrogenation of C18:3n-3 and FA composition in milk. Water was freely available during the entire experiment.

2.2. Diets

The control diet was prepared as a TMR which consisted of grass silage (600 g/kg DM), corn silage (100 g/kg DM), concentrates (240 g/kg DM) and linseed (60 g/kg DM). In the sainfoin diet, half of the grass silage DM was replaced by a sainfoin silage mixture. The characteristics of the silages are included in Table 1. Dietary preparation, feed samples, feed analyses were described by Huyen et al. (2016).

Diet formulations (Table 1) were identical for both experimental periods. Diets were formulated to meet the energy and protein requirements of dairy cows (Van Es, 1975; Van Duinkerken et al., 2011) and to provide similar amounts of C18:3n-3 (Table 2). Each cow was fed ad libitum twice daily at 06:00 and 16:00 for 7 d before each experimental period. During the 29-d experimental period, diets were offered in 2 equal portions at a rate of 95% of ad libitum intake, which was determined during the 7-d period to minimize feed residues.

2.3. Measurements and sampling

During the last 4 d (d 26 to 29), data on feed offered, feed residues, milk production were recorded daily for each cow. However, the data of feed intake and milk production were of the same as reported by Huyen et al. (2016). Cows were milked twice daily 06:00 and 16:00. Representative milk samples (5 g/kg of milk) were taken at each milking time during the experimental period, pooled per cow per period and stored at −20 °C pending further analyses for FA.

2.4. Reticular digesta sampling

The digesta flow into the reticulum was assessed by the double marker method (Faichney, 1975; France and Siddons, 1986), using Cr-ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) for the liquid-phase and Yb-acetate for the particulate phase. Cr-EDTA was prepared by mixing Cr (III) chloride hexahydrate ($CrCl_3 \cdot 6H_2O$, equivalent to 2.20 g of pure Cr) dissolved in 800 mL and EDTA (17.57 g) dissolved in 200 mL of demineralized water. This solution (1 L) was heated to 100 °C for 2 h under continuous stirring. After cooling, pH was adjusted to 6.5 by adding NaOH (1 mol/L) and the volume was adjusted to 1 L with demineralized water. Yb-acetate was obtained from a commercial source (Dasico A/S, Birkerod, Denmark). Yb-acetate (equivalent to 1.5 g of pure Yb) was dissolved in 1 L of demineralized water under continuous stirring. The Cr-EDTA and Yb-acetate solutions were combined into a 2-L batch which was used for 1-d infusion into the rumen via the rumen cannula. Starting at d 26 of each experimental period, a primer doses of Cr-EDTA (equivalent to 3.3 g of pure Cr in 1-L solution) and Yb-acetate (equivalent to 2.25 g of pure Yb in 1-L solution) was infused into the rumen via the cannula over a 5-min period, in order to reach a rapid equilibrium of the ruminal marker

Table 1
Ingredients and chemical composition of diets used in the experiment (g/kg DM, Huyen et al., 2016).

Item	Dietary treatment		Grass silage	Sainfoin Zeus silage	Sainfoin Esparcette silage	Corn silage	Concentrate	Linseed
	Control	Sainfoin						
Ingredients								
Grass silage	600.0	300.0	–	–	–	–	–	–
Sainfoin silage[1]	0.0	300.0	–	–	–	–	–	–
Corn silage	100.0	100.0	–	–	–	–	–	–
Concentrate[2]	240.0	240.0	–	–	–	–	–	–
Linseed	60.0	60.0	–	–	–	–	–	–
Chemical composition								
DM, g/kg product	444.9	357.2	366.0	200.0	380.0	314.0	893.0	922.0
OM	918.9	891.3	907.1	785.2	923.5	961.3	916.3	962.0
CP	162.7	171.9	145.9	212.3	96.5	83.4	209.9	239.5
NDF	395.7	359.1	508.6	346.0	441.0	354.9	221.2	201.3
ADF	236.7	244.5	306.3	305.3	336.5	203.3	122.5	156.2
ADL	18.6	35.0	14.3	67.0	59.6	7.4	29.4	29.1
Crude fat[3]	37.8	35.1	0.0	0.0	0.0	0.0	40.3	417.9
Starch[3]	97.9	90.9	0.0	0.0	0.0	328.5	244.4	14.3
GE, MJ/kg DM	19.5	19.0	19.2	17.1	18.2	19.0	18.2	27.8
NE_L[4], MJ/kg DM	7.6	6.8	7.4	4.3	5.3	6.9	7.4	11.7
Condensed tannins	0.0	8.8	0.0	24.0	31.0	0.0	0.0	0.0

[1] Sainfoin silage was a mixture between cultivar Zeus silage from clay soil and cultivar Esparcette from sandy soil (the ratio between silages from cultivar Zeus and Esparcette = 70:30 on DM basis).
[2] Concentrate composition: triticale 3.4%, palm kernel flakes 11.8%, stable rapeseed 7.4%, rapeseed meal 7.2%, soybean meal 12.9%, beet pulp 7.5%, lime 1.53%, magnesium oxide 0.1%, mixing salt 0.42%, molasses 5%, sodium bicarbonate 0.25%, corn gluten middling 8.9%, corn 30.3%, potatoes juice 0.2% (protaminase), premix-vitamin 3.1%.
[3] The concentrations of crude fat and starch in grass silage, sainfoin Zeus silage, and sainfoin Esparcette silage were too low, so in the current study, the value was presented as 0.0, but the actual values have been included for calculation.
[4] Net energy for lactation (NE_L) was calculated according to Van Es (1975).

Table 2
Fatty acid composition of diets and diet ingredients used in the experiment (g/kg DM).

Fatty acid	Dietary treatment		Grass silage	Sainfoin Zeus silage	Sainfoin Esparcette silage	Corn silage	Concentrate	Linseed
	Control	Sainfoin						
C12:0	1.66	1.53	0.06	0.00	0.00	0.00	6.11	0.00
C14:0	0.61	0.58	0.00	0.00	0.14	0.00	2.29	0.00
C16:0	4.05	3.98	2.90	3.05	2.72	4.11	3.27	16.75
C16:1	0.25	0.24	0.44	0.39	0.30	0.00	0.00	0.00
C18:0	0.99	1.00	0.27	0.47	0.39	0.57	0.65	9.21
Cis-9-C18:1	5.36	5.00	0.28	0.23	0.49	5.18	5.59	47.60
Cis-9, cis-12-C18:2	6.76	6.27	2.14	1.34	2.49	13.11	6.50	36.65
Cis-9, cis-12, cis-15-C18:3	13.63	12.03	8.01	4.69	3.73	1.54	0.58	132.78
UFA[1]	26.00	23.54	10.87	6.65	7.02	19.83	12.67	217.03
Total FA[2]	33.31	30.62	14.10	10.17	10.28	24.51	24.98	242.98

[1] Unsaturated fatty acids (UFA) = Σ(C16:1, cis-9-C18:1, cis-9, cis-12-C18:2, cis-9, cis-12, cis-15-C18:3).
[2] Total fatty acids (FA) = Σ(C12:0, C14:0, C16:0, C16:1, C18:0, cis-9-C18:1, cis-9, cis-12-C18:2, cis-9, cis-12, cis-15-C18:3).

concentrations (Sterk et al., 2012b). Immediately after infusing the primer doses, the Cr-EDTA and Yb-acetate solution was infused into the rumen via the cannula at a constant rate (83.3 mL/h) for 4 d (d 26 to 29) using peristaltic pumps (BVP standard delivery pump, ISM 444, ISMATEC SA, Glattbrugg, Switzerland). For each day, a new 2-L batch markers solution was prepared. Marker infusions were continued until the last reticular digesta sample was collected on d 29 of each experimental period.

Reticular digesta samples (1 L) were obtained 3 times per day at 6-h intervals with the 1st, 2nd and 3rd samples at 09:00, 15:00, 21:00 on d 28 and the 4th, 5th and 6th samples at 06:00, 12:00, 18:00 on d 29, respectively, using the reticular sampling technique described by Krizsan et al. (2010). Briefly, a 250-mL wide-necked empty plastic bottle with a plastic stopper was manually placed in the reticulum through the rumen cannula, the plastic stopper was removed and refitted after the bottle was full, after which the bottle was removed from the reticulum. This process was repeated 4 times before the 1-L reticular digesta sample was manually filtered through a 1-mm sieve and the particles retained on the sieve were discarded. The sieved sample was immediately frozen and stored at −20 °C pending further analysis.

The reticular digesta samples was thawed at room temperature and pooled per cow per period, then filtered (by squeezing) through 2 layers of cheesecloth. The filtrate was centrifuged at 10,000 × *g* for 10 min at 4 °C and the collected pellet added to the solid matter retained on the cheesecloth (particulate phase). The supernatant phase after centrifugation was defined as the liquid phase. Liquid and particulate phase samples were stored frozen then they were freeze-dried and ground in a cross-beater mill (Peppink 100 AN, Deventer, The Netherlands) to pass through a 1-mm sieve before being stored at 4 °C until analysis of FA, Cr and Yb.

2.5. Analytical procedures

FA composition of feed stuffs, milk and reticular digesta samples were analyzed according to Folch et al. (1957), Khan et al. (2011). Briefly, FA in 375 mg of feed ingredient or reticular digesta samples were extracted with 15 mL of chloroform-methanol (2:1, vol/vol), containing internal standard (C13:0, 3 mg of C13:0 per 20 mL of chloroform-methanol) according to Folch et al. (1957). Fatty acids were methylated with 0.5-mol/L NaOH methanolate ($NaOCH_3$), followed by 6-mol/L HCl in methanol, and collected in hexane.

Hexane was then evaporated and the fatty acid methyl esters (FAME) were resuspended in 1 mL of hexane and were quantified by using gas chromatography (GC). For milk FA analysis, total lipids were extracted by centrifugation at 3,000 × *g* for 30 min at 4 °C. Total lipids were cleaned by heating at 60 °C in an oven for 10 min, followed by centrifugation (20,000 × *g*, 5 min, 20 °C). The clear lipids were dried using Na_2SO_4. Fatty acids from milk lipids were methylated with 30% of $NaOCH_3$, neutralized with $NaHSO_4$ and dried using Na_2SO_4. FAME were quantitatively transferred into a 1.5-mL GC vial. The FAME were quantified by using GC. The results of FA were expressed in gram per kilogram for individually feed ingredient in TMR samples and gram per 100 g of total FA for milk samples.

Cr was oxidized by wet-destruction as described by Pellikaan et al. (2013) and measured using an atomic absorption spectrophotometer (AA240FS, Varian BV, Middelburg, The Netherlands). Yb concentrations were determined by carbonization at 550 °C, followed by combustion at 550 °C as described by Sterk et al. (2012b). After cooling to room temperature, the ash was destructed in diluted nitric acid and Yb measured by inductively coupled plasma atomic emission spectrometry (ICP-AES; PerkinElmer Optima 3300 DV ICP; PerkinElmer, Groningen, the Netherlands).

2.6. Calculations

Fatty acid reticular inflow was calculated from the double marker method of Faichney (1975) described in France and Siddons (1986), using Cr-EDTA for the liquid-phase and Yb-acetate for the particulate phase. The relative proportions of the liquid and particulate phase in digesta were reconstituted based on the maker concentration in the liquid and particulate phase. The reconstitution factor (R_F) was calculated based on Eq. (1):

$$R_F = (C_{Yb,X} / I_{Yb} - C_{Cr,X} / I_{Cr}) / (C_{Cr,F} / I_{Cr} - C_{Yb,F} / I_{Yb}) \quad (1)$$

where $C_{Yb,X}$ and $C_{Cr,X}$ are the concentrations of Yb and Cr in the particulate phase, $C_{Yb,F}$ and $C_{Cr,F}$ are the concentrations of Yb and Cr in the liquid phase (mg Yb or Cr per g fresh weight [FW], FW is the weight before samples were freeze dried), I_{Yb} and I_{Cr} are the amounts of Yb and Cr infused in the rumen per day (mg/d).

The obtained R_F was used to calculate the flow of true reticular digesta based on Eq. (2):

$$\begin{aligned} F_D &= I_{Cr} \times (1 + R_F) / (C_{Cr,X} + R_F \times C_{Cr,F}) \\ &= I_{Yb} \times (1 + R_F) / (C_{Yb,X} + R_F \times C_{Yb,F}) \end{aligned} \quad (2)$$

where F_D is the amount of true reticular digesta flow per day (g FW/d).

The concentration of FA of true reticular digesta were calculated based on Eq. (3):

$$C_{Nutrient,D} = (C_{Nutrient,X} + R_F \times C_{Nutrient,F}) / (1 + R_F) \quad (3)$$

where $C_{Nutrient,D}$ is the concentration of FA of true reticular digesta (mg/g FW); $C_{Nutrient,X}$ is the concentration of FA of particulate phase (mg/g FW); $C_{Nutrient,F}$ is the concentration of FA of liquid phase (mg/g FW).

The FA reticular inflow per day were calculated based on Eq. (4):

$$F_{Nutrient} = C_{Nutrient,D} \times F_D \quad (4)$$

where $F_{Nutrient}$ is the amount of FA flow into reticular per day (mg/d).

Apparent rumen biohydrogenation of cis-9-C18:1, cis-9, cis-12-C18:2, cis-9, cis-12, cis-15-C18:3 and transfer efficiency of UFA feed to milk were obtained by using Eq. (5) and (6):

$$\text{Apparent rumen biohydrogenation (\%)} = 100 - [\text{UFA reticular inflow (g/d) / UFA intake (g/d)}] \times 100 \quad (5)$$

$$\text{Transfer efficiency of UFA feed to milk (\%)} = [\text{UFA in milk (g/d) / UFA intake (g/d)}] \times 100 \quad (6)$$

2.7. Statistical analysis

Effects of diet on FA intake, FA reticular inflow and milk FA composition were tested by analyses of variance using the MIXED procedure of SAS (2010). The statistical model used to analyze the data was as follows: $Y = \mu + A_i + T_j + P_k + \varepsilon_{ijk}$, where Y is the dependent variable; μ is the overall mean; A_i is the effect of cow ($i = 1$ to 6); T_j is the effect of diet treatment ($i = 1$ to 2); P_k is the effect of period ($k = 1$ to 2); and ε_{ijk} is the residual error term. In the model the independent variables treatment and period were included as fixed effects, with cow considered as a random variable. The data are presented as the least square of means and standard error of the means (LSM ± SEM). Differences among main effects were analyzed using Tukey-Kramer's multiple comparison procedure in the LSMEANS statement in SAS (2010). The effects considered significant was at a probability value of $P < 0.05$ and a trend at $0.05 \leq P < 0.10$.

3. Results

3.1. Fatty acids composition of diets, fatty acid intake and fatty acids flow

The fatty acid composition of the control and sainfoin diets are presented in Table 2. In general, the saturated fatty acids (SFA, C12:0, C14:0, C16:0, C18:0) composition was similar between the 2 diets, whereas the unsaturated FA (UFA, cis-9-C18:1, cis-9, cis-12-C18:2 and cis-9, cis-12, cis-15-C18:3) concentrations were numerically lower in the sainfoin diet compared to the control diet. The cis-9, cis-12, cis-15-C18:3 intake was lower (224.50 vs. 242.10 g/d; $P = 0.025$) in the sainfoin diet (Table 3), whereas the intake of other SFA and UFA did not differ between the 2 diets.

Total FA reticular inflow was not different ($P = 0.265$) between the 2 diets (Table 4). The odd and branched chain fatty acids (OBCFA) reticular inflow tended ($P = 0.098$) to be greater for cows fed the sainfoin diet. The MUFA reticular inflow was greater ($P = 0.042$) in cows fed the sainfoin diet. The reticular inflow of trans-9, trans-12-C18:2 and cis-12, trans-10 C18:2 was greater ($P \leq 0.024$) in the sainfoin diet group, whereas the PUFA reticular inflow was not different between the 2 diets. The UFA reticular inflow tended ($P = 0.080$) to be greater in the sainfoin diet group. The MUFA, PUFA and UFA reticular inflow were affected by period ($P \leq 0.033$).

3.2. Extent of biohydrogenation

The cows fed sainfoin diet had a lower ($P \leq 0.038$) apparent ruminal biohydrogenation of cis-9-C18:1 and C18:3n-3, compared to the cows fed the control diet (Table 5). Moreover, the apparent ruminal biohydrogenation of cis-9, cis-12-C18:2 tended ($P = 0.085$) to be lower in the sainfoin diet group. Apparent ruminal biohydrogenation was affected by period ($P \leq 0.028$).

Table 3
Fatty acid intake (g/d) of lactating dairy cows fed either control or sainfoin diet.

Fatty acid intake	Dietary treatment		SEM	*P*-value	
	Control	Sainfoin		Treatment	Period
C12:0	29.52	28.50	1.617	0.218	0.672
Anteiso-C13:0	0.86	0.43	0.111	0.002	0.001
C14:0	10.82	10.83	0.610	0.953	0.397
Iso-C15:0	0.00	0.40	0.092	0.001	0.001
C16:0	71.80	74.28	4.163	0.303	0.127
Anteiso-C16:0	7.50	6.51	0.387	0.001	0.005
OBCFA[1]	8.36	7.34	0.435	0.004	0.004
C16:1	4.40	4.42	0.267	0.911	0.067
C18:0	17.62	18.70	1.048	0.123	0.088
Cis-9-C18:1	95.02	93.33	5.315	0.539	0.037
Cis-9, cis-12-C18:2	119.77	117.20	6.723	0.512	0.003
Cis-9, cis-12, cis-15-C18:3	242.10	224.50	12.968	0.025	0.479
UFA[2]	461.29	439.49	25.218	0.122	0.038
Total FA[3]	599.43	579.14	33.077	0.246	0.053

[1] Odd and branched fatty acids (OBCFA) = Σ(anteiso-C13:0, iso-C15:0, anteiso-C16:0).
[2] Unsaturated fatty acids (UFA) = Σ(C16:1, cis-9-C18:1, cis-9, cis-12-C18:2, cis-9, cis-12, cis-15-C18:3).
[3] Total fatty acids (FA) = Σ(C12:0, C14:0, C16:0, OBCFA, C16:1, C18:0, cis-9-C18:1, cis-9, cis-12-C18:2, cis-9, cis-12, cis-15-C18:3).

3.3. Milk fatty acid profile

Milk fatty acid profiles of lactating dairy cows are shown in Tables 6 and 7. The sainfoin diet group had greater ($P \leq 0.018$) C18:3n-3 and cis-9, cis-12-C18:2 proportions in the milk FA profile compared to the control diet group. Consequently, the proportions of PUFA in the milk FA profile were greater ($P = 0.013$) for the sainfoin diet than for the control diet. The higher ($P = 0.006$) proportion of total trans-C18:1 in the sainfoin diet group was caused by the higher ($P \leq 0.019$) proportion of trans-9-C18:1, trans-11-C18:1, trans-12-C18:1, trans-13, trans-14-C18:1 and trans-15-C18:1. In addition, the sainfoin diet group had higher ($P \leq 0.031$) proportions of cis-11-C18:1 and cis-12-C18:1 compared to the control diet group. However, the total proportion of SFA and UFA in the milk FA profile did not differ between the 2 diets.

Transfer efficiencies from feed to milk of total C18:2, C18:3n-3 and UFA were greater (Table 8; $P \leq 0.0179$) for cows on the sainfoin diet. Feeding the sainfoin diet for dairy cows increased the transfer efficiency from feed to milk of C18:3n-3 and UFA with 1.47% ($P = 0.0013$) and 14.52% ($P = 0.0179$), respectively, compared to the control diet.

4. Discussion

4.1. Fatty acids reticular inflow

The higher content of MUFA reticular inflow in the sainfoin diet group is consistent with the result reported by Vasta et al. (2009a), who supplemented 4.7% (based on DM diet) tannin from quebracho extract to herbage or concentrate fed sheep. They observed a 10% greater ruminal MUFA concentration for the herbage diet and a 75% greater ruminal MUFA concentration for the concentrate diet, compared to those without supplementation of tannin (Vasta et al., 2009a). Moreover, a 7% greater ruminal PUFA concentration for the herbage diet and a 62% greater PUFA concentration for the concentrate diet were found when tannin was added to the diet.

In a rumen simulation technique (RUSITEC) study, Khiaosa-Ard et al. (2009) supplemented grass-clover hay with 7.9% (based on DM diet) tannin extract from *Acacia mearnsii*. They found a 162% greater ruminal trans-11-C18:1 and a 45% greater ruminal cis-11-C18:1 concentration for the grass-clover hay diet with addition of tannin, compared to the diet without tannin. In the current study, the sainfoin diet contained 8.8 g of CT/kg diet DM. The cows fed sainfoin diet had a greater MUFA reticular inflow than those fed control diet. However, there was no difference in stearic acid reticular inflow between the 2 diets. Based on the present results and those of Khiaosa-Ard et al. (2009), we suggest that CT may have inhibited the last step of biohydrogenation, the reduction of C18:1 to stearic acid.

Table 4
Fatty acid reticular inflow (g/d) of lactating dairy cows fed either control or sainfoin diet.

Item	Dietary treatment		SEM	*P*-value	
	Control	Sainfoin		Treatment	Period
SFA					
C12:0	7.39	8.93	0.763	0.185	0.544
C13:0	43.13	52.72	4.214	0.088	0.003
C14:0	10.92	13.22	1.032	0.106	0.238
C15:0	7.18	8.17	0.827	0.255	0.099
C16:0	123.82	143.34	11.737	0.094	0.108
C17:0	2.51	3.03	0.257	0.046	0.102
C18:0	399.11	409.92	34.301	0.731	0.189
C20:0	3.25	4.30	0.357	0.015	0.179
C22:0	4.87	5.17	0.497	0.627	0.377
C24:0	3.67	4.60	0.514	0.211	0.175
Iso-C14:0	0.82	1.00	0.179	0.491	0.139
Anteiso-C14:0	0.65	1.22	0.486	0.285	0.132
Iso-C15:0	3.28	3.62	0.444	0.609	0.797
Anteiso-C15:0	7.67	7.68	0.907	0.988	0.620
Iso-C16:0	2.35	2.95	0.364	0.194	0.194
Anteiso-C16:0	2.55	3.28	0.447	0.185	0.336
Iso-C17:0	1.47	1.27	0.222	0.402	0.023
OBCFA[1]	71.63	84.93	7.353	0.098	0.006
SFA[2]	624.68	674.35	55.245	0.331	0.085
MUFA					
C16:1	2.40	2.78	0.291	0.357	0.717
Trans-9-C18:1	2.45	4.08	0.249	0.003	0.789
Cis-9-C18:1	33.31	40.39	4.134	0.059	0.023
MUFA[3]	38.18	47.25	4.529	0.042	0.033
PUFA					
Trans-9, trans-12-C18:2	1.94	3.37	0.485	0.024	0.043
Cis-9, cis-12-C18:2	13.56	16.29	1.631	0.199	0.025
CLA, cis-12, trans-10-C18:2	1.83	2.82	0.285	0.015	0.049
CLA, cis-9, trans-11-C18:2	12.48	11.78	1.500	0.535	0.452
Cis-9, cis-12, cis-15-C18:3	12.39	15.64	1.466	0.110	0.042
PUFA[4]	42.20	49.90	4.833	0.142	0.032
UFA[5]	80.35	97.15	9.313	0.080	0.031
Total FA[6]	705.07	771.48	63.333	0.265	0.071

SEM = standard error of means.
[1] Odd and branched chain fatty acids (OBCFA) = Σ(C13:0, C15:0, C17:0, iso-C14:0, anteiso-C14:0, iso-C15:0, anteiso-C15:0, iso-C16:0, anteiso-C16:0, iso-C17:0).
[2] Saturated fatty acids (SFA) = Σ(C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C24:0, OBCFA).
[3] Mono-unsaturated fatty acids (MUFA) = Σ(C16:1, trans-9-C18:1, cis-9-C18:1).
[4] Poly-unsaturated fatty acids (PUFA) = Σ(trans-9, trans-12-C18:2, cis-9, cis-12-C18:2, CLA, cis-12, trans-10-C18:2, CLA, cis-9, trans-11-C18:2, cis-9, cis-12, cis-15-C18:3).
[5] Unsaturated fatty acids (UFA) = Σ(MUFA, PUFA).
[6] Total fatty acids (FA) = Σ(SFA, UFA).

Table 5
Apparent ruminal biohydrogenation (%) of fatty acid in lactating dairy cows fed either control or sainfoin diet.

Item	Dietary treatment		SEM	*P*-value	
	Control	Sainfoin		Treatment	Period
Cis-9-C18:1	65.4	56.3	3.08	0.038	0.012
Cis-9, cis-12-C18:2	88.6	85.5	1.14	0.085	0.006
Cis-9, cis-12, cis-15-C18:3	94.9	93.0	0.44	0.035	0.028

SEM = standard error of the mean.

Table 6
Milk fatty acid profile (g/100 g) of lactating dairy cows fed either control or sainfoin diet.

Item	Dietary treatment		SEM	*P*-value	
	Control	Sainfoin		Treatment	Period
SFA					
C4:0	3.94	3.97	0.329	0.954	0.010
C6:0	2.59	2.53	0.200	0.780	0.026
C8:0	1.64	1.36	0.139	0.148	0.009
C10:0	3.25	2.88	0.225	0.099	0.007
C11:0	0.19	0.12	0.076	0.525	0.506
C12:0	4.06	3.65	0.228	0.092	0.021
C14:0	12.58	11.87	0.249	0.096	0.045
C15:0	1.06	0.91	0.060	0.117	0.092
C16:0	24.67	24.58	0.601	0.799	0.006
C17:0	0.45	0.45	0.047	0.961	0.567
C18:0	12.49	12.56	0.423	0.844	0.005
C20:0	0.05	0.13	0.039	0.151	0.045
Iso-C15:0	0.29	0.24	0.042	0.441	0.208
Anteiso-C15:0	0.43	0.37	0.029	0.193	0.035
Iso-C16:0	0.20	0.26	0.031	0.191	0.061
Iso-C17:0	0.34	0.33	0.052	0.877	0.912
BCFA[1]	1.25	1.20	0.121	0.757	0.128
SFA[2]	68.25	66.22	1.431	0.120	0.071
MUFA					
C14:1	1.18	0.89	0.138	0.088	0.074
C16:1	1.61	1.54	0.158	0.645	0.722
C17:1	0.18	0.16	0.038	0.758	0.585
Trans-9-C18:1	0.33	0.47	0.033	0.015	0.176
Trans-11-C18:1	1.18	1.80	0.168	0.006	0.166
Trans-12-C18:1	0.36	0.49	0.038	0.0003	0.144
Trans-13 + 14-C18:1	0.88	1.23	0.081	0.019	0.024
Trans-15-C18:1	0.69	0.89	0.048	0.009	0.067
Trans-16+Cis-14-C18:1	0.61	0.67	0.043	0.273	0.629
Total trans-C18:1[3]	4.06	5.55	0.371	0.006	0.116
Cis-9-C18:1	21.24	21.45	0.996	0.774	0.030
Cis-11-C18:1	0.39	0.44	0.032	0.015	0.330
Cis-12-C18:1	0.27	0.40	0.044	0.031	0.385
Cis-13-C18:1	0.07	0.07	0.017	0.882	0.010
Cis-15-C18:1	0.40	0.44	0.027	0.101	0.272
Total cis-C18:1[4]	22.37	22.80	1.048	0.580	0.038
MUFA[5]	29.39	30.94	1.342	0.201	0.078
PUFA					
Trans-9, trans-12-C18:2	0.37	0.39	0.019	0.386	0.099
Cis-9, cis-12-C18:2	0.90	1.04	0.041	0.011	0.110
Total non-conjugated C18:2[6]	1.27	1.43	0.055	0.016	0.519
CLA, cis-9, trans-11 C18:2	0.45	0.55	0.055	0.136	0.670
Cis-9, cis-12, cis-15-C18:3	0.64	0.86	0.063	0.018	0.251
PUFA[7]	2.36	2.84	0.155	0.013	0.318
UFA[8]	31.75	33.78	1.431	0.120	0.071

[1] Branched chain fatty acids (BCFA) = Σ(iso-C15:0, anteiso-C15:0, iso-C16:0, iso-C17:0).
[2] Saturated fatty acids (SFA) = Σ(C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, BCFA).
[3] Total trans-C18:1 = Σ(trans-9-C18:1, trans-11-C18:1, trans-12-C18:1, trans-13 + 14-C18:1, trans-15-C18:1, trans-16+cis-14-C18:1).
[4] Total cis-C18:1 = Σ(cis-9-C18:1, cis-11-C18:1, cis-12-C18:1, cis-13-C18:1, cis-15-C18:1).
[5] Mono-unsaturated fatty acids (MUFA) = Σ(C14:1, C16:1, C17:1, total trans-18:1, total cis-18:1).
[6] Total non-conjugated C18:2 = Σ(trans-9, trans-12-C18:2, cis-9, cis-12-C18:2).
[7] Poly-unsaturated fatty acids (PUFA) = Σ(total non-conjugated C18:2, CLA, cis-9, trans-11 C18:2, cis-9, cis-12, cis-15-C18:3).
[8] Unsaturated fatty acids (UFA) = Σ(MUFA, PUFA).

Total FA leaving the rumen in the current study were higher (105.64 g/d for the control diet and 192.34 g/d for the sainfoin diet) than total FA intake in both diets, an observation in line with the study of Doreau and Ferlay (1994). This result can be explained by the synthesis of FA from volatile FA by ruminal bacteria and protozoa. Indeed, bacteria contain 10% to 15% lipids in dry matter (Viviani, 1970). Another reason is FA catabolism requires aerobic conditions and the rumen is an anaerobic environment, hence little catabolism can be expected to have occurred. In addition, the rate of absorption of FA in the rumen is very low (Noble, 1978) resulting in the majority of dietary FA flowing into the reticulum.

The lower extent of rumen biohydrogenation of the sainfoin fed cows could be caused by the CT in sainfoin diet. In an in vitro study, Vasta et al. (2009c) reported that tannins reduced ruminal biohydrogenation by the inhibition of ruminal microorganism rather than by a direct interaction of tannins with the enzymes involved in the biohydrogenation pathway. Jones et al. (1994) found that CT from sainfoin inhibited the growth of *B. fibrisolvens*, one of the bacteria species involved in ruminal biohydrogenation.

In addition, the lower extent of rumen biohydrogenation could also be related to the level of NDF present in the diet. Sackmann et al. (2003) reported that biohydrogenation proceeds at a higher level with increasing NDF content in the diet. The microorganisms which are involved in biohydrogenation are mainly cellulolytic bacteria, such as *B. fibrisolvens*, which are more abundantly present in fiber rich diets (Kepler and Tove, 1967). Vasta et al. (2009a) also found that in lambs fed herbage, the ruminal environment was more favorable for the process of biohydrogenation than in lambs fed concentrate. In the current study, the greater extent of rumen biohydrogenation for cows fed the control diet could be explained in part by the higher dietary NDF content, compared to the sainfoin diet. Fiber fermentation produces acetate and butyrate, the biochemical pathways which liberate 2 H^- ions (Tavendale et al., 2005), and these are used in rumen biohydrogenation. The rumen biohydrogenation in the current study ranged from 56.3% to 94.9%. A previous study of Sterk et al. (2012b) reported apparent rumen biohydrogenation levels ranging from 73.5% to 98.5%. Supplementation of vegetable oils, oilseeds or tannin in dairy cow diets could affect the rumen biohydrogenation level. The rumen biohydrogenation level could be lower when tannin was supplemented into dairy cow diets. In the current study, the effect of period on the MUFA, PUFA and UFA reticular inflow and apparent ruminal biohydrogenation could be due to the effect of lactation stage in dairy cows (Stoop et al., 2009). These authors observed a lower concentration of UFA in mid-lactation compared to early and late lactation dairy cows. In addition, the concentration of conjugated linoleic acid (CLA, cis-9, trans-11-C18:2) increased with lactation stage (Stoop et al., 2009). The milk fatty acid profile affected by lactation stage could, however, not be explained by milk fat percentage that linearly increases from 4.24% to 5.02% from d 100 to 300 of lactation (Stoop et al., 2009). In the current study, the UFA intake ($P = 0.038$) and total FAs intake ($P = 0.053$) were also affected by the period. This could be a cause for the effect of period on the MUFA, PUFA and UFA reticular inflow and apparent ruminal biohydrogenation.

4.2. Fatty acids profile in milk

The increase in milk PUFA in the sainfoin diet group could be explained in part by the lower ruminal biohydrogenation in this diet. Although C18:3n-3 intake was lower in cows fed the sainfoin diet, compared to the control diet, the concentration of PUFA, especially cis-9, cis-12-C18:2 and C18:3n-3 in milk fat were greater in cows receiving the sainfoin diet. The transfer efficiency from feed to milk of C18:3n-3 in cows fed the sainfoin diet was correspondingly increased. The current results are in agreement with findings of Henke et al. (2017) and Kälber et al. (2013). Kälber et al. (2013) replaced ryegrass silage by buckwheat silage, which contained total tannin at 7.6 g/kg DM in dairy cow diets. Their results showed that milk fat was richer in PUFA, especially C18:2n-6 and C18:1n-9, although C18:3n-3 intake of cows fed the buckwheat diet was lower than cows fed the ryegrass diet. The apparent recovery of dietary C18:3n-3 in milk was greater in the buckwheat diet,

Table 7
Milk fatty acid profile (g/d) of lactating dairy cows fed either control or sainfoin diet.

Item	Dietary treatment		SEM	*P*-value	
	Control	Sainfoin		Treatment	Period
SFA					
C4:0	43.34	43.53	7.561	0.979	0.047
C6:0	27.85	28.12	4.757	0.945	0.084
C8:0	18.08	14.90	3.109	0.330	0.046
C10:0	34.47	31.31	4.974	0.423	0.063
C11:0	1.76	1.37	0.691	0.699	0.200
C12:0	43.37	39.65	5.462	0.393	0.160
C14:0	131.60	129.73	12.855	0.823	0.553
C15:0	11.64	9.92	1.517	0.347	0.321
C16:0	260.95	274.94	32.813	0.086	0.006
C17:0	4.74	5.08	0.789	0.657	0.319
C18:0	133.31	141.15	17.696	0.066	0.001
C20:0	0.54	1.56	0.474	0.167	0.035
Iso-C15:0	3.11	2.68	0.597	0.619	0.239
Anteiso-C15:0	4.56	4.03	0.580	0.452	0.149
Iso-C16:0	2.13	2.85	0.539	0.305	0.171
Iso-C17:0	3.57	3.73	0.783	0.878	0.917
BCFA[1]	13.38	13.28	2.238	0.976	0.307
SFA[2]	725.02	734.59	89.392	0.791	0.996
MUFA					
C14:1	11.73	9.50	0.707	0.058	0.048
C16:1	16.47	16.70	1.835	0.878	0.530
C17:1	1.84	1.59	0.398	0.673	0.577
Trans-9-C18:1	3.43	4.90	0.302	0.018	0.401
Trans-11-C18:1	12.07	19.74	1.789	0.005	0.061
Trans-12-C18:1	3.76	5.34	0.523	0.004	0.606
Trans-13 + 14-C18:1	9.08	13.73	1.187	0.020	0.019
Trans-15-C18:1	7.05	9.86	0.770	0.007	0.028
Trans-16+Cis-14-C18:1	6.36	7.38	0.726	0.054	0.130
Total trans-C18:1[3]	41.75	60.96	4.83	0.004	0.028
Cis-9-C18:1	217.80	234.08	16.941	0.109	0.008
Cis-11-C18:1	3.90	4.68	0.194	0.015	0.560
Cis-12-C18:1	2.71	4.34	0.464	0.014	0.969
Cis-13-C18:1	0.74	0.738	0.135	0.994	0.009
Cis-15-C18:1	4.22	4.73	0.422	0.033	0.888
Total cis-C18:1[4]	229.36	248.57	17.358	0.067	0.008
MUFA[5]	301.15	337.33	22.103	0.009	0.003
PUFA					
Trans-9, trans-12-C18:2	3.82	4.25	0.319	0.043	0.446
Cis-9, cis-12-C18:2	9.21	11.39	0.739	0.016	0.075
Total non-conjugated C18:2[6]	13.03	15.64	1.034	0.018	0.159
CLA, cis-9, trans-11 C18:2	4.50	5.98	0.574	0.117	0.385
Cis-9, cis-12, cis-15-C18:3	6.57	9.33	0.793	0.019	0.150
OBCFA[7]	33.35	31.25	3.81	0.465	0.368
PUFA[8]	24.10	30.95	2.132	0.019	0.155
UFA[9]	325.25	368.28	23.605	0.007	0.004
Total milk fat	1050.28	1102.87	112.09	0.191	0.199

[1] Branched chain fatty acids (BCFA) = Σ(iso-C15:0, anteiso-C15:0, iso-C16:0, iso-C17:0).
[2] Saturated fatty acids (SFA) = Σ(C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, BCFA).
[3] Total trans-C18:1 = Σ(trans-9-C18:1, trans-11-C18:1, trans-12-C18:1, trans-13 + 14-C18:1, trans-15-C18:1, trans-16+cis-14-C18:1).
[4] Total cis-C18:1 = Σ(cis-9-C18:1, cis-11-C18:1, cis-12-C18:1, cis-13-C18:1, cis-15-C18:1).
[5] Mono-unsaturated fatty acids (MUFA) = Σ(C14:1, C16:1, C17:1, total trans-18:1, total cis-18:1).
[6] Total non-conjugated C18:2 = Σ(trans-9, trans-12-C18:2, cis-9, cis-12-C18:2).
[7] Odd and branched chain fatty acids (OBCFA) = Σ(C11:0, C15:0, C17:0, C17:1, BCFA).
[8] Poly-unsaturated fatty acids (PUFA) = Σ(total non-conjugated C18:2, CLA, cis-9, trans-11 C18:2, cis-9, cis-12, cis-15-C18:3).
[9] Unsaturated fatty acids (UFA) = Σ(MUFA, PUFA).

Table 8
Transfer efficiency (%) of C18:2, C18:3n-3 and UFA from feed to milk of lactating dairy cows fed either control or sainfoin diet.

Item	Dietary treatment		SEM	Treatment	Period
	Control	Sainfoin			
OBCFA	395.01	423.36	32.414	0.510	0.127
Total C18:2	7.64	9.75	0.315	0.0015	0.0136
C18:3n-3	2.68	4.15	0.215	0.0013	0.0622
UFA	70.98	85.50	3.748	0.0179	0.0218

OBCFA = odd and branched chain fatty acids; UFA = unsaturated fatty acids.

compared with the ryegrass diet (Kälber et al., 2013). In the rumen environment, a very large part (>90%) of the C18:3n-3 intake is biohydrogenated (Vasta et al., 2009a). Thus, even small changes in the biohydrogenation rate of C18:3n-3 will lead to large effects on the concentrations of PUFA in milk fat (Jayanegara et al., 2011).

CT have been shown to inhibit the last step of biohydrogenation (Khiaosa-Ard et al., 2009), which may explain the accumulation of trans-11-C18:1 in the sainfoin diet group, compared to the control diet group in the current study. The results of current study are in agreement with literature. Dschaak et al. (2011) reported that total

trans-C18:1 and C18:3n-3 in bovine milk FA increased with quebracho CT extract supplementation at 30 g/kg DM. The increase in proportion of trans-11-C18:1, cis-9, cis-12-C18:2 and C18:3n-3 in milk fat were found in dairy ewes fed a control diet plus a mixture of tannin extract at 10 g/kg DM (Toral et al., 2011). Sainfoin pellets fed to lactating cows resulted in an increasing proportion of C18:3n-3 (17%) in milk and cheese fat, compared to milk and cheese fat in cows fed the basal diet (Girard et al., 2015).

In relation to human health, there is a matter of debate that ruminant trans-FA raise the risk for cardiovascular diseases in comparison with non-ruminant industrially derived (Catherine et al., 2009). However, vaccenic acid (trans-11-C18:1) accounts for about 50% to 80% of the total trans-FA content in ruminant milk fat (Lock et al., 2004). Vaccenic acid is metabolized into CLA (cis-9, trans-11-C18:2). The CLA is known as a beneficial nutrient for human health because it is associated with the prevention of allergy and asthma (Katrin et al., 2016). In the current study, the concentration of total trans-C18:1 was greater ($P = 0.006$) in milk fat of cows fed the sainfoin diet. This result can be considered as unbeneficial from a human nutritional health perspective. However, the increased concentrations of vaccenic acid (trans-11-C18:1), and PUFA (especially cis-9, cis-12-C18:2 and C18:3n-3) in milk fat of cows fed a sainfoin diet can be considered desirable for human health.

5. Conclusions

Replacing 50% of grass silage with sainfoin silage resulted in a higher unsaturated FA reticular inflow and a lower extent of ruminal biohydrogenation. Cows fed sainfoin diet improved concentration of vaccenic acid and PUFA in milk, especially cis-9, cis-12-C18:2 and C18:3n-3.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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References

Aufrère J, Dudilieu M, Andueza D, Poncet C, Baumont R. Mixing sainfoin and lucerne to improve the feed value of legumes fed to sheep by the effect of condensed tannins. Animal 2013;7:82–92.
Catherine JF, Heather HB, Spencer P, Donna V. Human health benefits of vaccenic acid. Appl Physiol Nutr Metabol 2009;34:978–91.
Doreau M, Ferlay A. Digestion and utilisation of fatty acids by ruminants. Anim Feed Sci Technol 1994;45:379–96.
Dschaak CM, Williams CM, Holt MS, Eun JS, Young AJ, Min BR. Effects of supplementing condensed tannin extract on intake, digestion, ruminal fermentation, and milk production of lactating dairy cows. J Dairy Sci 2011;94:2508–19.
Elgersma A, Ellen G, Van der Horst H, Muuse BG, Boer H, Tamminga S. Comparison of the fatty acid composition of fresh and ensiled perennial ryegrass (*Lolium perenne L.*), affected by cultivar and regrowth interval. Anim Feed Sci Technol 2003;108:191–205.
Faichney GJ. The use of markers to partition digestion within the gastro-intestinal tract of ruminants. In: McDonald IW, Warner ACI, editors. Digestion and metabolism in ruminants. Armidale: The University of New England Publishing Unit; 1975. p. 277–91.
Folch J, Lees M, Sloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957;226:497–509.
France J, Siddons RC. Determination of digesta flow by continuous market infusion. J Theor Biol 1986;121:105–19.
Girard M, Dohme-Meier FD, Wechsler D, Goy D, Kreuzer M, Bee G. Ability of 3 tanniferous forage legumes to modify quality of milk and Gruyère-type cheese. J Dairy Sci 2015;99:205–20.
Glasser F, Ferlay A, Chilliard Y. Oilseed lipid supplements and fatty acid composition of cow milk: a meta-analysis. J Dairy Sci 2008;91:4687–703.
Harfoot CG, Hazlewood GP. Lipid metabolism in the rumen. In: The rumen microbial ecosystem. The Netherlands: Springer; 1997. p. 382–426. ISBN: 978–94–009–1453–7.
Hatew B, Stringano E, Mueller-Harvey I, Hendriks WH, Hayot Carbonero C, Smith LMJ, Pellikaan WF. Impact of variation in structure of condensed tannins from sainfoin (*Onobrychis viciifolia*) on in vitro ruminal methane production and fermentation characteristics. J Anim Physiol Nutr 2016;100:348–60.
Hayot Carbonero C, Mueller-Harvey I, Brown TA, Smith LMJ. Sainfoin (*Onobrychis viciifolia*): a beneficial forage legume. Plant Genet Resour 2011;9:70–85.
Henke A, Westreicher-Kristen E, Molkentin J, Dickhoefer U, Knappstein K, Hasler M, Susenbeth A. Effect of dietary quebracho tannin extract on milk fatty acid composition in cows. J Dairy Sci 2017;100:6229–38.
Hoste H, Torres-Acosta JFJ, Sandoval-Castro CA, Mueller-Harvey I, Sotiraki S, Louvandini H, Thamsborg SM, Terrill TH. Tannin containing legumes as model for nutraceuticals against digestive parasites in livestock. Vet Parasitol 2015;212:5–17.
Huyen NT, Desrues O, Alferink SJJ, Zandstra T, Verstegen MWA, Hendriks WH, Pellikaan WF. Inclusion of sainfoin (*Onobrychis viciifolia*) silage in dairy cow rations affects nutrient digestibility, nitrogen utilization, energy balance, and methane emissions. J Dairy Sci 2016;5:3566–77.
Jayanegara A, Kreuzer M, Wina E, Leiber F. Significance of phenolic compounds in tropical forages for the ruminal bypass of polyunsaturated fatty acids and the appearance of biohydrogenation intermediates as examined in vitro. Anim Prod Sci 2011;51:1127–36.
Jones GA, McAllister TA, Muir AD, Cheng KJ. Effects of sainfoin *(Onobrychis viciifolia Scop.)* condensed tannins on growth and proteolysis by four strains of ruminal bacteria. Appl Environ Microbiol 1994;60:1374–8.
Kälber T, Kreuzer M, Leiber F. Effect of feeding buckwheat and chicory silages on fatty acid profile and cheese-making properties of milk from dairy cows. J Dairy Res 2013;80:81–8.
Katrin K, Christian D, Gerhard J. Evaluation of the impact of ruminant trans fatty acids on human health: important aspects to consider. Crit Rev Food Sci Nutr 2016;56:1964–80.
Kepler CR, Tove SB. Biohydrogenation of unsaturated fatty acids III. Purification and properties of a linoleate Δ^{12}–cis, Δ^{11}–trans-isomerase from Butyrivibrio fibrisolvens. J Biol Chem 1967;242:5686–92.
Khan NA, Cone JW, Pellikaan WF, Khan MA, Struik PC, Hendriks WH. Changes in fatty acid content and composition in silage maize during grain filling. J Sci Food Agric 2011;91:1041–9.
Khiaosa-Ard R, Bryner SF, Scheeder MRL, Wettstein HR, Leiber F, Kreuzer M. Soliva CR Evidence for the inhibition of the terminal step of ruminal α-linolenic acid biohydrogenation by condensed tannins. J Dairy Sci 2009;92:177–88.
Kraiem K, Garrett JE, Meiske JC, Goodrich RD, Marten GC. Influence of method of forage preservation on fibre and protein digestion in cattle given lucerne, birdsfoot trefoil and sainfoin. Anim Prod 1990;50:221–30.
Krizsan SJ, Ahvenjärvi S, Volden H, Broderick GA. Estimation of rumen outflow in dairy cows fed grass silage-based diets by use of reticular sampling as an alternative to sampling from the omasal canal. J Dairy Sci 2010;93:1138–47.
Lock AL, Corl BA, Barbano DM, Bauman DE, Ip C. The anticarcinogenic effect of trans-11 18:1 is dependent on its conversion to cis-9, trans-11 CLA by delta9-desaturase in rats. J Nutr 2004;134:2698–704.
Loor JJ, Ferlay A, Ollier A, Doreau M, Chilliard Y. Relationship among trans and conjugated fatty acids and bovine milk fat yield due to dietary concentrate and linseed oil. J Dairy Sci 2005;88:726–40.
McMahon LR, Majak W, McAllister TA, Hall JW, Jones GA, Popp JD, Cheng KJ. Effect of sainfoin on in vitro digestion of fresh alfalfa and bloat in steers. Can J Anim Sci 1999;79:203–12.
Noble RC. Digestion, absorption and transport of lipids in ruminant animals. Prog Lipid Res 1978;17:55–91.
Pellikaan WF, Verstegen MWA, Tamminga S, Dijkstra J, Hendriks WH. $\delta^{13}C$ as a marker to study digesta passage kinetics in ruminants: a combined in vivo and in vitro study. J Anim 2013;7:754–67.
SAS. SAS/STAT software. NC: SAS Institute Inc Cary; 2010. version 9.3.
Sackmann JR, Duckett SK, Gillis MH, Realini CE, Parks AH, Eggelston RB. Effects of forage and sunflower oil levels on ruminal biohydrogenation of fatty acids and conjugated linoleic acid formation in beef steers fed finishing diets. J Anim Sci 2003;81:3174–81.
Scharenberg A, Arrigo Y, Gutzwiller A, Soliva CR, Wyss U, Kreuzer M, Dohme F. Palatability in sheep and in vitro nutritional value of dried and ensiled sainfoin

(*Onobrychis viciifolia*) birdsfoot trefoil (*Lotus corniculatus*), and chicory (*Cichorium intybus*). Arch Anim Nutr 2007;61:481–96.
Shingfield KJ, Ahvenjärvi S, Toivonen V, Vanhatalo A, Huhtanen P, Griinari JM. Effect of incremental levels of sunflower-seed oil in the diet on ruminal lipid metabolism in lactating cows. Br J Nutr 2008;99:971–83.
Sivakumaran S, Molan AL, Meagher LP, Kolb B, Foo LY, Lane GA, Attwood GA, Fraser K, Tavendale M. Variation in antimicrobial action of proanthocyanidins from Dorycnium rectum against rumen bacteria. Phytochemistry (Oxf) 2004;65:2485–97.
Sterk A, Van Vuuren AM, Hendriks WH, Dijkstra J. Effects of different fat sources, technological forms and characteristics of the basal diet on milk fatty acid profile in lactating dairy cows–a meta-analysis. J Agric Sci 2012a;150:495–517.
Sterk A, Vlaeminck B, Van Vuuren AM, Hendriks WH, Dijkstra J. Effects of feeding different linseed sources on omasal fatty acid flows and fatty acid profiles of plasma and milk fat in lactating dairy cows. J Dairy Sci 2012b;95:3149–65. 2012b.
Stoop WM, Bovenhuis H, Heck JML, Van Arendonk JAM. Effect of lactation stage and energy status on milk fat composition of Holstein-Friesian cows. J Dairy Sci 2009;92:1469–78.
Tavendale MH, Meagher LP, Pacheco D, Walker N, Attwood GT, Sivakumaran S. Methane production from in vitro rumen incubations with *Lotus pedunculatus* and *Medicago sativa*, and effects of extractable condensed tannin fractions on methanogenesis. Anim Feed Sci Technol 2005;123:403–19.
Toral PG, Hervás G, Bichi E, Belenguer Á, Frutos P. Tannins as feed additives to modulate ruminal biohydrogenation: effects on animal performance, milk fatty acid composition and ruminal fermentation in dairy ewes fed a diet containing sunflower oil. Anim Feed Sci Technol 2011;164:199–206.
Van Duinkerken G, Blok MC, Bannink A, Cone JW, Dijkstra J, Van Vuuren AM, Tamminga S. Update of the Dutch protein evaluation system for ruminants: the DVE/OEB2010 system. J Agric Sci 2011;149:351–67.
Van Es A. Feed evaluation for dairy cows. Livest Prod Sci 1975;2:95–107.
Vasta V, Mele M, Serra A, Scerra M, Luciano G, Lanza M, Priolo A. Metabolic fate of fatty acids involved in ruminal biohydrogenation in sheep fed concentrate or herbage with or without tannins. J Anim Sci 2009a;87:2674–84.
Vasta V, Priolo A, Scerra M, Hallett KG, Wood JD, Doran O. Δ^9 desaturase protein expression and fatty acid composition of longissimus dorsi muscle in lambs fed green herbage or concentrate with or without added tannins. Meat Sci 2009b;82:357–64.
Vasta V, Makkar HPS, Mele M, Priolo A. Ruminal biohydrogenation as affected by tannins in vitro. Br J Nutr 2009c;102:82–92.
Vasta V, Yáñez-Ruiz DR, Mele M, Serra A, Luciano G, Lanza M, Biondi L, Priolo A. Bacterial and protozoal communities and fatty acid profile in the rumen of sheep fed a diet containing added tannins. Appl Environ Microbiol 2010;76:2549–55.
Viviani R. Metabolism of long-chain fatty acids in the rumen. Adv Lipid Res 1970;8:267–346.