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Suitability of Sainfoin (*Onobrychis viciifolia*) Hay as a Supplement to Fresh Grass in Dairy Cows

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ABSTRACT : Two experiments were carried out to determine the utility of sainfoin hay, a temperate tanniferous forage legume, as a dietary supplement for grass-fed cows. The condensed tannins (CT) of sainfoin might counteract the typical metabolic ammonia load of cows in intensive grazing systems. Furthermore, the physical fibrousness of sainfoin might improve ruminal pH stability. In the preliminary experiment, the eating rate of non-lactating Holstein cows of two tanniferous hays, sainfoin and birdsfoot trefoil, was compared to that of a grass-clover hay after specific periods of time (n = 4). The eating rate of sainfoin was superior to that of the other forages. In the main experiment, designed as a replicated 3×3 Latin square, six ruminally-cannulated, lactating Red Holstein cows received grass, concentrate and either no supplementation, 3 kg/d of grass hay or 3 kg/d of sainfoin hay (n = 6). Measured intakes of the grass hay and the sainfoin hay were 2.0 and 1.5 kg DM, and two cows entirely refused to eat the sainfoin hay and had to be excluded from data analysis. Grass DMI was similar for cows supplemented with sainfoin hay and cows fed only grass whereas intake of concentrate was higher ($p<0.01$) for the latter treatment. Continuous measurement of ruminal pH showed that the minimum pH at night tended to be lower ($p<0.10$) with grass-only feeding compared to sainfoin supplementation, but pH did not decline below the threshold of subacute acidosis for a longer period of time. The slightly higher intake of nitrogen (N) for cows supplemented with sainfoin hay (413 g/d) compared to cows fed only grass (399 g/d) was accompanied by an increased ($p<0.05$) fecal N excretion and a tendency for an increased ($p<0.10$) urinary N excretion. Ruminal ammonia concentration, as well as plasma and milk urea, were not affected by sainfoin supplementation. In conclusion, the lack of positive effects typical for CT might be explained either by the limited CT content of this plant species (55 g/kg DM) or the relatively low proportion of sainfoin in the total diet or both. Moreover, due to the unexpected low grass quality, the general ammonia load might have been too low for CT to have an impact. (**Key Words :** Condensed Tannins, Dairy Cows, Nitrogen Balance, Ruminal pH, Sainfoin)

INTRODUCTION

Pasture grass from intensive grazing systems is characterized by high concentrations of rapidly degradable protein (van Vuuren et al., 1991) and carbohydrates (O'Grady et al., 2008) as well as low concentrations of physical effective fiber (O'Grady et al., 2008). Due to the fast ruminal degradation of protein, ammonia is available after feeding at an excessive level and can not be utilized to a sufficient amount by the ruminal microbes. Therefore, ammonia has to be metabolized by the liver to urea, causing liver stress. This metabolic pathway is also energy consuming, and nitrogen (N) is lost through urine. Also, high concentrations of ammonia in the blood may impair fertility (Visek, 1984). The high content of rapidly degradable carbohydrates, in turn, increases the yield of VFA, which may reduce ruminal pH in grazing cows to an acidotic level (O'Grady et al., 2008), particularly when combined with a reduced ruminal buffering capacity. The latter depends on saliva flow and, consequently, chewing activity, which may be impaired when cows are consuming feed with a low physical fibrousness (Balch, 1971). Furthermore, ingestion of feed with a high moisture content, which is characteristic for fresh grass (van Vuuren et al., 1991), was described to be associated with less saliva production (Meyer et al., 1964).

New ways of supplementary feeding might help to respond to these challenges. Condensed tannins (CT) are known to reduce ruminal protein degradability, and may increase fecal N excretion (Hervas et al., 2004). Sainfoin (*Onobrychis viciifolia*) is a temperate forage legume which

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has, compared to other temperate forages, a moderate to high content of CT (Scharenberg et al., 2007a). When fed as hay to sheep, sainfoin showed a high palatability (Scharenberg et al., 2007a) and a reduced ruminal proteolysis at unchanged body N retention (Scharenberg et al., 2007b). An alternative temperate CT legume would be birdsfoot trefoil (*Lotus corniculatus*) (Scharenberg et al., 2007a). In addition to the CT-dependent property, legume hay is assumed to have the potential of balancing the above mentioned negative effects in cows grazing young grass as hay is supposed to have more physical fibrousness than fresh forage.

To our knowledge, only limited data exist on supplementing grass-fed cows with CT from temperate forages. Therefore, the hypothesis was tested that the tanniferous forage sainfoin, given as hay, has an advantage over a typical grass hay in counterbalancing the limitations young pasture grass has in dairy cows with respect to N metabolism, ruminal pH, and chewing behavior. A preliminary experiment should clarify which tanniferous legume, sainfoin or birdsfoot trefoil is better suitable as a CT alternative and whether they are sufficiently accepted by cows.

MATERIALS AND METHODS

Preliminary experiment

Six non-lactating, ruminally cannulated Holstein cows (BW: 634±39.8 kg) were subjected to a preliminary experiment consisting of two consecutive periods of 11 d duration each. Every cow was randomly subjected to two of the three treatments (incomplete Latin square; $n = 4$). During the first 7 d of each period, cows were adapted to the feeding schedule and were offered 4 kg of a poor quality hay composed of grasses, legumes and herbs (CP 60.1 g/kg DM; NDF, 654 g/kg DM) at 08:00 h and 11 kg (equivalent to *ad libitum* feeding) of the same hay at 11:00 h. During the last 4 d of each period, at the 08:00 h feeding time the hay supplied was replaced by either 4 kg of sainfoin hay (CP, 234 g/kg DM; NDF, 264 g/kg DM; CT, total, 96.2 g/kg DM, extractable, 66.4 g/kg DM, protein-bound, 24.1 g/kg DM, fiber-bound, 5.77 g/kg DM), birdsfoot trefoil hay (CP, 189 g/kg DM; NDF, 292 g/kg DM; CT, total, 28.7 g/kg DM, extractable, 20.7 g/kg DM, protein-bound, 6.31 g/kg/DM, fiber-bound, 1.72 g/kg/DM), or grass-clover hay (CP, 235 g/kg DM; NDF, 356 g/kg DM). Consumption of the experimental hay types was measured after 30, 90, and 240 min. On the last day of each period, ruminal fluid was sampled at 11:00 h to measure pH (pH electrode, no. 6.0220.100, Metrohm, Herisau, Switzerland; pH-meter, no. 692, Metrohm) and to analyze ammonia concentration.

Main experiment: design, animals, and feeds

In a replicated 3×3 Latin Square arrangement, six ruminally cannulated multiparous Red Holstein cows were blocked by milk yield and were randomly assigned to three treatments in three consecutive experimental periods, each lasting 21 d. At the beginning of the experiment, cows, on average, were 60.7±5.09 d in milk, had a BW of 626±41.5 kg, and produced 37.1±4.48 kg/d of milk. Each experimental period consisted of a 14-d adaptation period where cows were individually housed in a tie stall with rubber mat flooring, and a 7-d collection period where cows were kept in metabolism crates equipped with a slatted floor. For animal welfare reasons, cows were kept in a free-stall barn for one week each between the experimental periods resulting in a total of 11 wk of experiment. Prior to the beginning of the experimental periods, the animals got accustomed to the metabolism crates by putting them into the crates for one day.

All cows had *ad libitum* access to pasture grass in a zero grazing system. In the first treatment, cows received only grass in fresh form (GF), while in a second and third treatment, from 07:30 h to 10:30 h, 3 kg of either grass hay (GH) or sainfoin hay (SH) were offered instead of pasture grass. Assuming that 22 kg/d of milk with 4.0% fat and 3.2% protein can be produced from the estimated grass intake (ALP, 2007), cows were fed 0.5 kg/d ground and pelleted barley per kg of additionally produced milk. In order to produce these pellets the barley was mixed with 50 g/kg fat. Furthermore, all cows received 300 g/d of a mineral mixture designed for grass-fed cows (Graf et al., 2005) containing, per kg: 118 g Ca, 45.5 g P, 21.6 g Mg, 89.7 g Na, 1.24 g Zn, 475 mg Cu, 70 mg Se, 25 mg I, 5 mg Co. This mineral mixture was combined with 50 g/kg fat, 511 g/kg barley and 7.2 g/kg wheat middlings in order to facilitate the pelleting process and to ensure complete consumption. The mineral mixture and the barley were mixed and offered in two equal meals at 07:00 h and 16:30 h for half an hour. During this time cows had no access to the forage. Throughout the experiment, fresh water was available at all times.

The swards, where the pasture grass was taken from, had been already cut once in daily portions starting about 4 wk before the first experimental period was planned to begin in order to have young grass with a similar quality available throughout the experiment. However, due to unexpectedly dry and warm weather conditions, the realized re-growth periods differed to some extent with 33, 30 and 39 d in the three experimental periods, respectively. The average botanical composition of the grass harvested, as determined once weekly, was characterized by high proportions of grasses (82.5±9.17% dominated by *Lolium perenne*, *Phleum pretense*, *Poa annua*, *Festuca rubra*), fewer legumes (16.6±9.15%; *Trifolium repens*), and herbs (1.0±0.87%, *Taraxacum officinale*). The grass hay was of a first cut and harvested in the previous year from a ley. At

the time of cutting, the sward consisted of 72.9±6.08% grasses, 4.9±2.46% legumes and 22.2±4.75% herbs. The sainfoin was harvested as the second cut after 21 d of regrowth. At harvest, the sward consisted of 80.1% sainfoin and 15.4% dandelion (*Taraxacum officinale*). Immediately after mowing, the harvested material was dehydrated in a special device where 30°C warm air was forced in a closed system through the forages, dried in a condenser and warmed again. This procedure was used to obtain dried sainfoin with a high proportion of leaves (Scharenberg et al., 2007b), which are richer in CT than the stems (Häring et al., 2007).

Data recording and sample collection

During the collection period, milk yields were determined at each milking (05:30 h; 16:00 h). Milk samples of every milking were preserved with Broad Spectrum Microtabs (Gerber Instruments AG, Effretikon, Switzerland) for analysis of fat, protein, and lactose. Additionally, milk samples were pooled by cow across the entire collection period and stored at -20°C for later analysis of N and urea contents.

Grass, hays, pelleted barley and the mineral mixture were sampled every day. Grass was analyzed daily for DM by lyophilization. All feed samples were pooled across the entire collection periods for later analysis of chemical composition. Feed intake and water consumption were recorded daily and, at 08:30 h, total feces and urine were collected, weighed, sampled, and stored at -20°C. Urine was collected by the use of urinals attached around the vulva by Velcro straps glued to the shaved skin. The urine was collected in acidified form (3 M sulphuric acid) to avoid gaseous N losses. The daily samples of feces and urine were thawed and pooled proportionately across the collection periods for each cow and frozen again for later analysis.

From d 16 to d 21 of each experimental period during 22 h/d quasi continuous (every 30 sec) measurements of the ruminal pH were performed using a self-constructed device as described in detail by Graf et al. (2005). During the same 6 d, eating and ruminating behavior was individually recorded by registering jaw movements with the use of the IGER Behavior Recorder (Institute of Grassland and Environment Research, North Wyke, UK) as developed by Rutter et al. (1997). Records lasting for more than 22 h/d were used for analysis and extrapolated to 24 h. The data of the jaw movement recordings were read and analyzed automatically using the Microsoft Windows application 'Graze' (Rutter, 2000). The program discriminated time spent for eating, rumination and time without jaw movement during the day (07:00 to 19:00 h) and the night (19:00 to 07:00 h). Jaw movements lasting for less than 2 min and being away from the next movement by more than 1 min were defined as idle time in addition to the time where no movements were recorded.

On d 21 of each experimental period, ruminal fluid and blood samples were taken at 07:00 h, 11:00 h, 15:00 h, and 19:00 h. Blood was collected from the *vena jugularis* into vacuum tubes and cooled on ice until being centrifuged (Universal 16, Hettich, Tuttlingen, Germany) at 1,500×*g* for 15 min. Plasma and serum (only for analysis of NEFA) were sampled using either heparinized vacuettes® (Greier Bio-One, Solingen, Germany) or containers without anticoagulant. Ruminal fluid was taken from the ventral part of the rumen through a tube with a terminal cone. For ammonia and VFA determination, 5 ml of ruminal fluid were mixed with 0.1 ml 5% (w/v) trichloroacetic acid solution, and 10 ml of ruminal fluid were mixed with 0.2 ml 25% (w/v) sulphuric acid solution, respectively. Blood and ruminal fluid samples were stored at -20°C.

All procedures were in accordance with the Swiss guidelines for animal welfare and were approved by the Animal Care Committee of the Canton Fribourg, Switzerland.

Laboratory analysis

Prior to the laboratory analysis, feed samples and refusals were dried at 60°C for 12 h and ground to pass a 1.0-mm screen (Brabender mill, no. 880804, Brabender, Duisburg, Germany). Contents of DM of hays, pelleted barley, mineral mixture and refusals were quantified gravimetrically (3 h at 105°C) and that of the feces by lyophilization, grinding like the other samples, and subsequent drying at 105°C for 3 h. The nutrient contents of feeds, refusals, and lyophilized feces were analyzed by standard methods. Total ash was determined by incineration at 550°C for 4 h. Cell wall constituents were analyzed using the ANKOM 200/220 Fiber Analyzer (Ankom Technology Corporation, Fairport, NY, USA). Thereby, ADF was analyzed according to AOAC (1995, procedure 973.18) and was expressed without residual ash after incineration at 500°C for 1 h. Analysis of NDF based on Mertens (2002) with heat stable amylase and being expressed without residual ash. Physically effective NDF (peNDF) was calculated by multiplying the NDF contents of the forages with the proportion of particles >8 mm. For this purpose, a particle separator (Gorr GmbH & Co. KG, Eschwege, Germany) was used (cf. Dohme et al., 2007). Sugar content of the feeds was measured with a method based on Kunerth and Youngs (1984) adapted to an AutoAnalyzer II (Bran and Luebbe, Hamburg, Germany). Starch content of the barley and mineral mix was analyzed by the polarimetric method (procedure 7.21; AOAC, 1995). Total N content of feeds, refusals, feces, milk and urine was analyzed by the Kjeldahl procedure (988.05; AOAC, 1995). The CT content

of the forages was analyzed applying the butanol-HCl method as described by Terrill et al. (1992). This method additionally provides the opportunity to distinguish between the acetone-water extractable CT, the CT still bound to the plant protein after the first extraction process and a fiber-bound fraction of CT, which remains after the extraction of the first two fractions. *Lotus pedunculatus* was used as a standard for all CT measurements, and measurements were done on an UV/VIS Spectrometer (PerkinElmer, Schwerzenbach, Switzerland). In the incineration residues of the feed, calcium (Ca), magnesium (Mg), sodium (Na), and potassium (K) were quantified after solubilisation in nitric acid (65%) with an inductive coupled plasma optical emission spectrometer (ICP-OES, Optima 2000 DV, Perkin-Elmer, Schwerzenbach, Switzerland).

In ruminal fluid, ammonia concentration was analyzed colorimetrically with a commercial test-kit (Coffret Urea-Kit S 180, bioMérieux, Geneva, Switzerland). Determination of VFA was performed based on the method of Niven et al. (2004) on a HPLC (System HPLC, Dionex, Sunnyvale, USA) equipped with an integrated column (Nucleogel ION 300 OA 300×7.8 mm, Macherey-Nagel, Düren, Germany).

In urine and milk, urea was analyzed after enzymatic treatment with urease and glutamate dehydrogenase (Urea-Kit UV 250, bioMérieux) on an autoanalyser (COBAS Mira, Roche Diagnostics, Rotkreuz, Switzerland; standard: Calimat, bioMérieux). The preserved milk samples were analyzed for contents of fat, protein, and lactose (FIL-IDF, 2000; method 141C) by infrared spectrometry (Combis-Foss, Gerber Instruments AG, Effretikon, Switzerland).

Blood plasma was analyzed for concentrations of glucose (test kit no. 1447513, Roche, Basle, Switzerland), NEFA (in blood serum; FA 115, Randox, Crumlin, UK), β-hydroxy butyrate (BHB; Ranbut RB 1008, Randox), total protein (no. 1553836, Roche), albumin (no. 1553836, bioMérieux), urea (no. 61974, bioMérieux), creatinine (Jaffé, Roche) and aspartate aminotransferase (ASAT; Enzyline ASAT/GOT 20 monoréactif', bioMérieux).

Calculations and statistical analysis

The dietary contents of absorbable protein at the duodenum and NE_L were calculated according to ALP (2007).

For evaluation of the continuously obtained pH data, the day was separated into a diurnal (07:00 to 19:00 h) and a nocturnal (19:00 to 07:00 h) period. For these periods, minimum, maximum, and mean pH values, as well as the time when pH was below 6.0 and 5.8 were calculated. These benchmarks were chosen because the activity of ruminal cellulolytic bacteria is compromised when ruminal pH drops below 6.0 (Mould et al., 1983) and the incidence of subacute acidosis increase when ruminal pH falls below 5.8 (Beauchemin et al., 2003). Eating, rumination, and idle time were similarly divided into the diurnal and the nocturnal period.

The results of the preliminary experiment were evaluated with the MIXED procedure of SAS (SAS Institute, 2004) with treatment as fixed effect (n = 4). In the main experiment, data of two cows had to be excluded from treatment SH because they refused to eat the sainfoin hay. Therefore, data were analyzed by an incomplete Latin square design using the MIXED procedure. Treatment and period, were considered fixed effects, and cow as random effect. A Latin square design with the repeated statement was used for the blood and ruminal fluid variables sampled four times on d 21 in each experimental period. Sampling time was included as the repeated factor. Because the interaction (treatment×sampling time) was mostly not significant, only the least square means of the main factors are presented in the tables and interactions are described in the text, if existent. Differences were tested using the PDIFF option. Significance was declared at $p<0.05$ and trends were discussed when $p<0.10$.

RESULTS

Preliminary experiment

From the beginning, the intake of the hay made from birdsfoot trefoil was lower ($p<0.001$) than that of the grass-

Table 1. Intake rates by dairy cows of hays made of grass-clover, sainfoin, birdsfoot trefoil and the effects on ruminal fluid variables in a preliminary experiment ($n = 4$)

	Grass-clover	Sainfoin	Birdsfoot trefoil	SEM	p-value
Feed intake (kg DM within min)					
30	2.84[a]	2.74[a]	1.22[b]	0.092	<0.001
90	3.06[a]	3.11[a]	1.85[b]	0.073	<0.001
240	3.12[b]	3.37[a]	2.89[c]	0.078	<0.001
Ruminal fluid variables					
pH	6.63	6.48	6.66	0.060	
Ammonia (mmol/L)	8.84[a]	6.05[b]	5.56[b]	0.556	<0.01

Within rows, values with different superscripts are significantly different at $p<0.05$.

clover hay and the sainfoin hay (Table 1). This intake difference became smaller in magnitude with time but remained significant ($p<0.001$). After 240 min, the intake was highest ($p<0.001$) for sainfoin hay followed by grass-clover hay and birdsfoot trefoil hay. The ruminal pH was not significantly affected by hay type. Ruminal ammonia concentration was lower ($p<0.01$) by 32 and 37% in sainfoin and birdsfoot trefoil hay, respectively, in comparison to the grass-clover hay. Based on these results, sainfoin was chosen as the test CT-legume for the main experiment.

Main experiment

Grass hay and sainfoin hay fed in the main experiment clearly differed in CP content (on DM basis), with a low CP content of the grass hay similar to the fresh grass (Table 2). The contents of NDF and peNDF were lower in sainfoin than in the fresh grass and the grass hay. The average sugar content of the sainfoin hay was lower than that of the other forages but contents varied largely among samples. The calculated contents of duodenally absorbable protein and NE_L in the fresh grass were lower than expected. Total CT content of the sainfoin hay was about 55 g/kg, whereof 61% were extractable, 29% bound to protein, and 10% bound to fiber. The CT content of the grass hay was negligible. Therefore, the analysis of the CT content in grass was omitted. The forages differed in mineral contents as well, especially Ca and Na. As expected, barley was characterized by a high starch content and, compared to the forages, a low content of minerals.

Cows in treatment GH had the highest ($p<0.05$) intake of total DM but the lowest intake of grass ($p<0.01$) compared to cows in treatment SH and GF (Table 3). The intake of barley and mineral mixture (concentrate) was similar for treatments GH and GF but lower ($p<0.01$) for treatment SH. Cows in treatment GH consumed more ($p<0.05$) hay compared to cows in treatment SH which resulted in a higher ($p<0.05$) intake of OM and NDF in GH compared to SH and GF. Water consumption was lowest ($p<0.001$) with the GF treatment followed by treatments SH and GH. The apparent OM digestibility was higher ($p<0.05$) in GH compared to SH whereas GF took an intermediate position. Milk fat percentage was lower ($p<0.05$) and lactose percentage tended to be higher ($p<0.1$) in GH compared to SH and GF (data not shown in table). However, these differences became non-significant when fat and lactose were expressed as daily yield. Milk yield, protein yield, protein percentage and urea were not influenced by

Table 2. Chemical composition (means±SD) of the experimental feeds (g/kg DM) as used in the main experiment (n = 3)

Item (g/kg DM)	Supplemented hays		Fresh grass	Barley supplement[1]
	Grass	Sainfoin		
DM (g/kg)	877±12.8	877±11.7	191±22.9	875±0.9
OM	919±8.9	900±6.9	911±6.7	981±0.2
CP	131±11.8	201±7.6	124±23.2	98±0.6
NDF	492±14.4	318±0.95	427±14.6	185±24.4
peNDF[2]	478±13.9	308±0.94	414±24.1	-[3]
ADF	277±3.0	261±1.96	265±23.4	52.5±7.89
Sugar	110.6±3.78	50.3±29.82	77.8±32.2	19.2±0.73
Starch	-	-	-	516±13.6
Calculated contents[4]				
Absorbable protein	92.0±2.65	132.3±2.52	93.3±5.51	94.3±0.58
NE_L (MJ/kg DM)	5.63±0.06	5.47±0.06	5.97±0.06	8.19±0.02
CT[5] (total)	0.9±0.67	54.8±1.19	-	-
Extractable	0.0±0.00	33.7±2.36	-	-
Protein-bound	0.6±0.95	15.8±0.24	-	-
Fiber-bound	0.4±0.29	5.3±0.93	-	-
Minerals				
Ca	3.20±0.260	12.37±0.516	6.25±1.706	0.47±0.018
Mg	1.49±0.074	2.29±0.150	2.09±0.510	1.12±0.023
Na	0.47±0.087	0.08±0.006	0.31±0.050	0.09±0.017
K	29.9±4.27	33.6±2.66	28.4±2.27	4.3±0.06

[1] Barley plus 50 g/kg fat. [2] peNDF = physically effective NDF. [3] Not analysed. [4] According to ALP (2007). [5] CT = Condensed tannins.

Table 3. Intake and apparent digestibility, milk yield and composition as well as nitrogen balance of cows in the main experiment (n = 6)

Item	Treatment[1]			SEM	p-value
	GF	GH	SH[2]		
Daily intake per cow (kg)					
Total DM	19.2[b]	19.8[a]	18.8[b]	1.01	<0.05
Grass DM	14.9[a]	13.1[b]	14.7[a]	0.43	<0.01
Concentrate[3] DM	4.3[a]	4.6[a]	2.8[b]	0.61	<0.01
Hay	-	2.0[a]	1.5[b]	0.19	<0.05
OM	17.6[b]	18.2[a]	17.1[b]	0.96	<0.05
NDF	7.71[b]	8.08[a]	7.69[b]	0.319	<0.05
ADF	4.48	4.63	4.65	0.171	
Tap water	44.4[c]	57.1[a]	51.4[b]	4.11	<0.001
Digestibility (%)					
OM	69.3[ab]	70.7[a]	67.8[b]	0.56	<0.05
NDF	65.9	66.4	64.9	0.85	
ADF	63.1	64.3	61.4	1.04	
Milk (kg/d)	26.2	27.3	25.7	1.81	
Fat (kg/d)	1.08	1.06	1.06	0.095	
Protein (kg/d)	0.82	0.86	0.80	0.075	
Lactose (kg/d)	1.28	1.37	1.26	0.093	
Urea (g/d)	4.15	4.33	4.91	0.328	
Nitrogen (N) balance (g/d)					
Intake	392[b]	409[a]	413[a]	20.5	<0.05
Faecal N excretion	152[b]	159[ab]	171[a]	10.8	<0.05
Urinary N excretion	106[(b)]	105[(b)]	127[(a)]	5.7	<0.10
N excretion with milk	130	135	127	12.1	
Body N retention	4.8	10.5	-18.0	16.44	
N balance (% of N intake)					
Faecal N	39.0[b]	38.8[b]	41.6[a]	0.78	<0.05
Urinary	26.7	25.3	30.3	1.62	
Milk N	35.2	34.9	33.3	3.56	
Urinary N (% of N excreted)	40.3	39.4	42.5	1.63	
Urinary urea (mmol/L)	97.6	91.7	104.9	10.13	

Within rows, values with different superscript are significantly different at p<0.05 and values with different superscripts in brackets tended to be different (p<0.10).

[1] GF = Grass as only forage in diet; GH = Grass supplemented with grass-clover hay; SH = Grass supplemented with sainfoin hay. [2] n = 4. [3] Barley supplement plus mineral mixture.

treatments. A slightly, but significantly, lower (p<0.05) intake of N was observed for cows fed GF compared to those fed GH and SH. Fecal N excretion was higher (p<0.05) with SH compared to GF resulting in the highest (p<0.05) proportion of fecal N in total N intake. Furthermore, urinary N excretion tended to be higher (p<0.10) when cows were fed SH instead of GF and GH. No differences were observed in concentration of urinary urea. Body N retention and N excretion with milk were also similar in all treatments.

The minimum ruminal pH at night (Table 4) tended to be higher (p<0.10) when SH was fed instead of GF and GH whereas mean and maximum ruminal pH and minimum ruminal pH at day were only numerically higher. Furthermore, the time periods when pH declined below 6.0 and 5.8 were numerically longer for the GF treatment compared to the other two treatments but these differences were not significant. Concerning eating patterns, no differences were observed among treatments in eating and ruminating time at day and at night. However, total idle time tended to be shorter (p<0.10) in treatment SH compared to GH. Treatment GF took an intermediate position.

Table 4. Ruminal pH and chewing activity as recorded across 6 days in the main experiment (n = 6)

Item	Treatment[1]			SEM	p-value
	GF	GH	SH[2]		
Ruminal pH					
Daytime (07:00-19:00 h)					
Mean	6.21	6.19	6.38	0.151	
Maximum	6.63	6.61	6.66	0.109	
Minimum	5.89	5.85	6.06	0.193	
Time pH <6.0 (min)	175	163	48	88.6	
Time pH <5.8 (min)	84	76	41	66.3	
Nocturnal (19:00-07:00 h)					
Mean	6.06	6.13	6.29	0.191	
Maximum	6.49	6.44	6.70	0.183	
Minimum	5.68$^{(b)}$	5.82$^{(b)}$	6.10$^{(a)}$	0.229	<0.10
Time pH <6.0 (min)	367	271	155	142.2	
Time pH <5.8 (min)	260	175	87	121.2	
Chewing activity (min/d)					
Daytime (07:00-19:00 h)					
Eating	343	324	361	13.0	
Ruminating	231	221	231	6.9	
Nocturnal (19:00-07:00 h)					
Eating	173	177	183	18.8	
Ruminating	341	329	343	12.2	
Total					
Eating	516	502	547	30.2	
Ruminating	572	550	574	16.3	
Time idle	361$^{(ab)}$	399$^{(a)}$	328$^{(b)}$	19.9	<0.10

Within rows, values with different superscripts in brackets tended to be different (p<0.10).
[1] GF = Grass as only forage in diet; GH = Grass supplemented with grass-clover hay; SH = Grass supplemented with sainfoin hay. [2] n = 4.

The GH cows had a lower (p<0.01) ruminal ammonia concentration than GF and SH cows (Table 5) whereas sampling time had no effect on ruminal ammonia level. Both concentration and profile of ruminal VFA were not influenced by dietary treatment, but followed a distinct pattern over the sampling times. Total VFA concentration tended to increase (p<0.10) from 07:00 to 11:00 h. Acetate proportion declined (p<0.001) over the day whereas propionate and *n*-butyrate proportions increased (p<0.05). There was a treatment×sampling time interaction (p<0.05) found for the proportion of *iso*-butyrate. This interaction can be explained by a slight increase at 19:00 h for treatment GF and GH compared to treatment SH where a linear decrease over the day was observed. Plasma glucose and creatinine tended to be highest (p<0.10) in GH fed cows and lowest in SH fed cows. Compared to the SH cows, plasma BHB level tended to be lower (p<0.10) in GF and GH cows whereas plasma urea level was lower (p<0.01) only in GH cows. The other plasma metabolites were not affected by treatment. Sampling time had an influence on NEFA, BHB and urea. The level of NEFA was highest (p<0.001) at 07:00 h while BHB remained constant until 15:00 h and then increased (p<0.001) until 19:00. Plasma urea declined from 11:00 h onwards (p<0.001).

DISCUSSION

There is an ongoing search for feeds suitable for supplementary feeding of cows offered fresh grass as their major forage. Such feeds have to be palatable to be consumed in the amounts intended. They have to balance a potential lack of the grass in physical fibrousness and, at the same time, the excessive metabolic N load resulting from the often high content of rapidly degradable protein in the grass. However, Bargo et al. (2003) reported in their review that supplementing grass-fed cows usually decreases grass DMI which is consistent with the intake behavior of cows in the main experiment when supplemented with grass hay

Table 5. Ruminal fluid variables and plasma metabolites and enzymes determined at different times on d 21 in the main experiment (n = 6)

Item	Treatment[1]			Sampling time (ST)				SEM	p-values	
	GF	GH	SH[2]	07:00	11:00	15:00	19:00		Treatment	ST
Ruminal fluid variables										
Ammonia (mmol/L)	4.13^{x}	2.79^{y}	4.90^{x}	3.99	3.42	3.88	4.47	0.544	<0.01	
VFA (mmol/L)	96.9	96.4	87.7	86.0$^{(b)}$	100.9$^{(a)}$	92.0$^{(ab)}$	95.7$^{(ab)}$	7.16		<0.10
VFA (molar %)										
Acetate	67.8	68.3	66.9	69.8^{a}	68.6^{a}	67.6^{a}	64.6^{b}	1.30		<0.001
Propionate	17.5	17.5	18.2	16.2^{b}	16.9^{ab}	17.2^{ab}	20.6^{a}	1.76		<0.05
n-Butyrate	11.5	11.3	11.6	10.7^{b}	11.4^{ab}	12.0^{a}	11.8^{a}	0.50		<0.05
iso-Butyrate	0.93	0.81	1.11	1.09	0.94	1.01	0.76	0.192		
n-Valerate	1.25	1.06	1.18	1.10	1.23	1.19	1.14	0.137		
iso-Valerate	1.04	1.06	1.01	1.12	0.95	1.04	1.03	0.139		
Plasma metabolites and enzymes[3]										
Glucose (mmol/L)	3.37$^{(xy)}$	3.52$^{(x)}$	3.26$^{(y)}$	3.49	3.41	3.32	3.30	0.117	<0.10	
NEFA (μmol/L)	106	131	104	181^{a}	91^{b}	89^{b}	95^{b}	22.1		<0.001
BHB (mmol/L)	0.54$^{(y)}$	0.53$^{(y)}$	0.62$^{(x)}$	0.54^{b}	0.54^{b}	0.53^{b}	0.66^{a}	0.032	<0.10	<0.001
Total protein (g/L)	82.8	83.6	82.1	83.9	82.6	82.3	82.6	1.11		
Albumine (g/L)	43.7	43.6	42.8	44.0	43.0	43.3	43.1	0.62		
Urea (mmol/L)	3.51^{x}	3.06^{y}	3.92^{x}	3.85^{a}	3.84^{a}	3.22^{b}	3.08^{b}	0.194	<0.01	<0.001
Creatinine (μmol/L)	100$^{(x)}$	101$^{(x)}$	95$^{(y)}$	100	98	98	98	2.22	<0.10	
ASAT (U/L)	77.4	77.0	75.7	75.8	74.9	78.2	77.7	2.07		

Within rows, treatment means (x, y, z) respectively sampling time means (a, b) with different superscript are significantly different at p<0.05 and values with different superscripts in brackets tended to be different (p<0.10).
[1] GF = Grass as only forage in diet; GH = Grass supplemented with grass-clover hay; SH = Grass supplemented with sainfoin hay. [2] n = 4. [3] ASAT = Aspartate aminotransferase; BHB = β-hydroxybutyrate.

but not of cows supplemented with sainfoin hay. As grass is the cheapest source of nutrients for ruminants (Clark and Kanneganti, 1998), it is necessary to find a balance between a maximal intake of grass and a beneficial intake of supplements.

Palatability

Condensed tannins may adversely affect voluntary intake (Barry and Duncan, 1984; Butter et al., 1999; Krebs et al., 2007) and selection (Villalba and Provenza, 2001) of such feeds in ruminants, which has been explained by possible interferences with saliva proteins in the mouth and the mucosa of the gastrointestinal tract and also because of the CTs' property to reduce protein digestibility. However, research so far has been performed mainly with sheep and there are known species differences in feed preference (Dumont and Petit, 1995). Additionally, effects on feeding behavior are likely to depend on the structure and properties of CT present in the forage (Mueller-Harvey, 2006).

Sainfoin is a temperate CT-containing legume with a relatively high nutritional value compared to other tanniferous temperate forages (Scharenberg et al., 2007a). It was shown to be without influence on feed intake of sheep after a short adaptation time (Scharenberg et al., 2007a). The results of the preliminary experiment suggest the same for cows. Furthermore, similar to the sheep (Scharenberg et al., 2007a), the cows exhibited a reduced feed intake when offered hay of birdsfoot trefoil, another temperate forage legume containing CT. In contrast to the experiment of Scharenberg et al. (2007a), where sheep had the choice between a tanniferous forage and a tannin-free control forage, in the preliminary experiment the eating rate of the tanniferous forage after specific periods of time was measured in cows. Similarly, the lactating cows of the main experiment had no access to another feed during the time when sainfoin was offered. Unexpectedly, the high voluntary intakes of non-lactating cows in the preliminary experiment could not be repeated, although the batch of sainfoin hay used in the main experiment was even lower in CT content than that of the preliminary experiment (55 vs. 96 g/kg DM). Two cows even completely refused the sainfoin hay. According to Provenza (1995), post-ingestive feedbacks may prompt ruminants to select the appropriate feed to meet their nutritional requirements that vary with age, physiological state, and environmental conditions, and at the same time to avoid toxicosis. For the present study, this would mean that lactating and non-lactating cows selected their feed in relation to different dietary aspects.

However, it still remains open whether or not the sainfoin intake would have differed when the non-lactating cows would have received exactly the same diet the lactating cows received and *vice versa*.

Ruminal pH

Cows fed high amounts of young, intensively grown pasture grass may approach subacute ruminal acidosis because this pasture grass has a high concentration of rapidly degradable carbohydrates and a lack of physical fibrousness (O'Grady et al., 2008). According to Beauchemin et al. (2003), the incidence of subacute ruminal acidosis increases when ruminal pH drops below 5.8. On the other hand, Plaizier (2004) used a ruminal pH of 6.0 as a threshold because activity of ruminal cellulolytic bacteria is already compromised below this pH level (Mould et al., 1983). This discrepancy shows that there is a disagreement about a precise definition of subacute ruminal acidosis and which ruminal pH depression is detrimental to the health and production of dairy cows. Schwartzkopf-Genswein et al. (2003) defined the incidence of subclinical ruminal acidosis in beef cattle as the situation when ruminal pH falls below 5.8 for more than 12 h/d. According to this definition, the cows in the present experiment did not suffer from ruminal acidosis. There was a trend for the minimum ruminal pH to be higher in cows supplemented with sainfoin hay compared to cows fed only grass or being supplemented with grass hay. However, this might be rather a result of the higher concentrate intake in the latter treatments than of insufficient supply of physically effective fiber because eating and in particular ruminating time were similar among treatment groups. One explanation for the missing of clear treatment effects on ruminal pH might be the poor grass quality due to the unfavorable weather conditions during the experiment. According to analyses, grass hay was richer in rapidly degradable carbohydrates and sainfoin hay contained less physically effective fiber than the fresh grass.

A greater efficiency of sainfoin over conventional hay in maintaining a high ruminal pH would require that sainfoin CT would slow down ruminal nutrient degradation. However, the influence of CT on the degradation of carbohydrates is still controversially discussed (Mueller-Harvey, 2006). In sheep, CT from sainfoin reduced the apparent digestibility of fiber in comparison to a tannin-free diet (treated with polyethylene glycol) (Scharenberg et al., 2007b). In the present study, no such effect of sainfoin CT was observed as can be seen from unchanged levels of fiber digestibility and ruminal VFA concentration compared to both the grass-only fed cows and the grass hay supplemented cows. Sainfoin hay had been carefully prepared in order to retain the tannin-rich leaves. This procedure, however, might be the reason why the contents of NDF and physical effective NDF of the sainfoin hay were much lower than those of the grass hay. Therefore, in situations where the main forage is really low in its physical fibrousness, carefully prepared sainfoin hay might be inferior to grass hay as a supplement.

Metabolic nitrogen load

In the preliminary experiment, there was a clear decline in ruminal ammonia concentration at similar intake of CP in cows fed sainfoin hay compared to grass-clover hay. This observation is in line with that of Scharenberg et al. (2007b) and indicates that CT of sainfoin were effective in reducing ruminal proteolysis of feed proteins. However, this effect could not be repeated in the main experiment, and ruminal ammonia as well as plasma und milk urea were similar in cows supplemented with sainfoin hay and those fed only grass. One explanation might be the comparably low ammonia load in comparison to other grazing studies (van Vuuren et al., 1993; Graf et al., 2005) preventing CT to be effective. Also the lower CT content of the sainfoin hay compared to that in the preliminary study is likely to have led to weaker effects. The 55 g CT/kg of the sainfoin hay used in the main experiment ranged between values found to be effective and those not effective. Accordingly, Min et al. (2003) reported that beneficial effects of CT in sheep can be expected with forages having CT concentrations of 20-45 g/kg DM, while Scharenberg et al. (2008) noted almost no effect with sainfoin hay having a content of 36 g/kg CT. By contrast, sainfoin hay and silage containing >70 g CT/kg DM (Scharenberg et al., 2007b) had a positive influence on N metabolism when fed to sheep. Barry and McNabb (1999) concluded from their experiments that beneficial effects of CT in a diet consisting of CT-containing and CT-free temperate forages can only be expected if the CT content is high (>90 g/kg DM) and the protein content relatively low in the CT-containing plant. However, they did not specify the ratio of CT-containing and CT-free forages in the diet. It is not clear whether in the main experiment one or both of the aforementioned factors were responsible for the lacking beneficial CT effects of sainfoin hay. Nevertheless, an increase in fecal N excretion, a typical effect of CT (Hervas et al., 2004; Waghorn, 2008), was observed in the present cows fed sainfoin hay. However, the anticipated simultaneous decrease in urinary N excretion (Scharenberg et al., 2007b; Waghorn, 2008) was missing. The increase of fecal N excretion is likely to be the result of an incomplete separation of proteins from CT as part of the complexes that have been dissolved at low pH in the abomasum might have been rebuilt in the lower at the high pH (Jones and Mangan, 1977). Alternatively, Kariuki and Norton (2008) suggested that such an increase in fecal N excretion is the result of an increased endogenous N excretion.

IMPLICATIONS

To our knowledge, this study is one of few studies investigating sainfoin in dairy cows. It showed that palatability is a very important item to be monitored. A high metabolic load, expected as a result of an extensive ruminal fermentation of dietary protein and rapidly degradable carbohydrates from pasture grass, failed to appear in the present study. The CT content of the sainfoin hay was medium and, due to the low intake, the sainfoin proportion in the total diet was low as well. Both may be responsible for the missing effects of CT on N metabolism. The lack of effect on ruminal pH and the low content of peNDF of sainfoin suggest that a supplementation strategy based on sainfoin will only be effective in young pasture grass of high quality and with sainfoin cultivars and harvesting strategies resulting in sufficiently high contents of peNDF and CT (maybe even >90 g/kg DM). The low acceptance of the sainfoin hay by the cows might be overcome by offering the supplement in another physical form such as pellets, but this processing method might reduce its physical fibrousness.

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