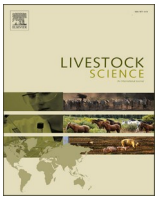
Livestock Science 248 (2021) 104509

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci



On-farm examination of sainfoin supplementation effects in dairy cows in a roughage-based feeding system: Indicators of protein utilisation

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HIGHLIGHTS

- An on-farm experiment assessed measures to improve N efficiency of grazing cows.
- Sainfoin was supplemented in applicable amounts intermittently or continously.
- Effects were clearer with intermittent compared to continuous supplementation
- Effects were too small to recommend sainfoin supplements for grazing cows

ARTICLE INFO

Keywords:
Cattle
Legume
Condensed tannins
Purine derivatives
Low-input
On-farm research

ABSTRACT

A case study was conducted with the objective to determine the effects of the tanniferous legume sainfoin (*Onobrychis viciifolia*) on protein utilisation of dairy cows, which were exposed to excessive dietary crude protein during autumn pasture grazing in a zero-concentrate feeding system. The study was conducted under practical conditions, deliberately accepting a certain lack of controllable variables. In order to assess a system applicable in practice, relatively small amounts of sainfoin were offered, and graded supply levels were compared by varying the duration of offer instead of dietary concentration. Within one dairy herd of 60 Swiss Fleckvieh cows, 30 individuals were chosen and randomly allocated to three groups balanced for milk yield, milk urea concentration, days in milk and parity. Over a period of 5 weeks, 2 kg per cow and day of sainfoin pellets were provided either intermittently, for 2 × 5 days (SST) in weeks 3 and 5 of the experiment or continuously over 35 days (SLT). A control group (CON) received 2 kg per cow and day of ryegrass pellets during the 5 weeks. The entire herd grazed on a protein-rich natural sward and was kept overnight in an open-space barn receiving fresh grass and hay *ad libitum*. Experimental pellets were offered individually twice per day in buckets. Feed, milk, faeces and urine samples were collected in weeks 0 (baseline), 3 and 5. Milk was analysed for fat, protein and urea, urine for purine derivatives, creatinine and nitrogen, and faeces for protein, fibre and particle fractions. During three sampling weeks, chewing activity was recorded by sensor halters. Treatment SST increased rumination time in the first half of the day, which was the only treatment effect on intake and rumination behaviour. Milk fat and protein yields were greater in SST compared to CON, but no treatment effect on concentration and yield of milk urea was found. The proportion of particle fractions in faeces was smaller in both sainfoin treatments compared to CON. In urine, the ratio of purine derivatives to creatinine tended to be higher in SST than in CON, and total urinary nitrogen excretion was lower, indicating a slightly better protein efficiency in SST. In summary, the study revealed small positive effects on protein metabolism and yield when sainfoin was applied short-term over two short periods, whereas long-term application had no effect.

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https://doi.org/10.1016/j.livsci.2021.104509
Received 24 May 2020; Received in revised form 28 March 2021; Accepted 7 April 2021
Available online 10 April 2021

1. Introduction

The nutrient composition of pasture grass changes with season, year and management. This means a varying diet with possible deficits or excesses in certain nutrients when cattle are fed exclusively with forages from grassland (Kuusela, 2004). Such imbalances may lead to problems in performance, health and fertility. An excess of crude protein (CP) on pasture in spring and autumn can be one of these problems (Pacheco and Waghorn, 2008). Due to the lack of energy and carbon skeletons, the high nitrogen (N) concentrations in pasture forages cannot be sufficiently used by the rumen microbes for their own protein synthesis (Sutter et al., 2017). Instead, ammonia produced during rumen CP degradation is directly absorbed through the rumen wall and metabolised in the liver to urea. Only a small part of this urea is recycled to the rumen; most is excreted with urine and milk (Reynolds and Kristensen, 2014). The detoxification of absorbed ammonia is an unnecessary energy loss to the cow (Parker et al., 1995) and stresses the liver. In addition, it increases the environmental impact of animal husbandry, because urinary N is more volatile than faecal N (Weiss et al., 2009; Chobtang et al., 2016). Commonly such imbalances in dietary energy and N sources are compensated by providing supplement feeds rich in energy but low in N, such as corn silage or concentrate. However, to prevent feed-food competition in animal production systems and to reserve arable land directly for food production (Schader et al., 2015), the omission of such feeds in cattle diets is being considered increasingly as an option (Leiber et al., 2017).

One way to achieve lower metabolic urea loads and urinary N excretion while at the same time improving protein use efficiency in dairy cows exclusively fed on grassland resources could be the inclusion of tannin-rich herbs in the diet (Totty et al., 2013). Tannins bind to proteins in the rumen and these bonds may partly be released in the abomasum (McNabb et al., 1998). Therefore, these proteins are either available in the small intestine for digestion or are excreted with faeces instead of urine (Dschaak et al., 2011). A promising tannin-containing herb growing under temperate climatic conditions is sainfoin (*Onobrychis viciifolia*). Sainfoin contains more than 60 different phenolic compounds (Regos et al., 2009), including various condensed tannins (CT) (Wang et al., 2015). When mixed with alfalfa, sainfoin tannins were found to lower solubility and, therefore, ruminal degradability of CP (Aufrere et al., 2013). Lower ruminal ammonia concentrations have been observed by Kraiem et al. (1990) when sainfoin was added to alfalfa diets of steers. Feeding sainfoin can reduce milk urea and urinary N excretion (Grosse Brinkhaus et al., 2016; Zhang et al., 2019); however, such effects are not found consistently (Scharenberg et al, 2009; Huyen et al., 2016), which may be due to varying dietary proportions of sainfoin and CT concentrations in the herbage.

In agricultural practice, high dietary inclusion levels of herbs are, depending on the species, limited by their high production costs, low palatability, moderate nutritive value and their possible anti-nutritional effects. At the same time, too low tannin supplementation levels may fail in achieving the desired effects (Scharenberg et al., 2009; Kapp-Bitter et al., 2020). Therefore, the positive effects of tannin-rich herbs at reasonable dietary inclusion levels have to be demonstrated under realistic conditions in practice. Given that dosage per day should not be reduced, duration may provide leverage to gain the desired effects at low cost. On the other hand, effects on ruminal ammonia formation may depend on the duration of herb feeding (Khiaosa-ard et al., 2012).

In the light of the existing studies on sainfoin supplements for dairy ruminants, which are often conducted under highly controlled conditions with large experimental effort, the questions arise, (i) whether sainfoin effects on nitrogen efficiency can be demonstrated under conditions prevailing in practice, (ii) whether the application of expensive sainfoin is effective in the short term too, and (iii) whether this can be detected with data, samples and analytical methods applicable on commercial farms. Against this background, an on-farm experiment with dairy cows grazing on autumn pasture in a zero-concentrate feeding system was designed. With the objective of counterbalancing expected dietary N excess, pelleted sainfoin was offered in feasible dosages, and the effects of a long-term versus short-term temporary offer on indicators of protein conversion efficiency and N excretion via urine and faeces were assessed. The particular challenge of conducting such an experiment under commercial on-farm conditions was addressed by choosing the best available proxies for a sound estimation of digestive and metabolic effects.

2. Materials and methods

2.1. Experimental design and protocol

An experiment (approved by the Cantonal Veterinary Office of Aargau, Switzerland; licence number AG75689) was carried out on a dairy farm with a zero-concentrate feeding system. The experiment lasted for 6 weeks from September to November 2016. After a baseline data collection week (week 0), 30 Swiss Fleckvieh cows were assigned for five weeks to three dietary treatments. These comprised a control treatment (CON) offering per cow 2 kg/day (as fed) of pelleted ryegrass (*Lolium perenne*) and two treatments with 2 kg/day (as fed) of pelleted sainfoin (*Onobrychis viciifolia*). In one sainfoin treatment (sainfoin short-term, 'SST'), the pellets were only fed during the first five days of weeks 3 and 5 (the two periods when data and sample collection took place). The other group received the sainfoin pellets over the entire five-week period (sainfoin long-term, 'SLT'). The ryegrass for pellet production was harvested after flowering and pelleted by Gebrüder Herzog (Hornussen, Switzerland). The sainfoin was harvested during the flowering stage, pelleted by and purchased from Agrobio Schönholzer AG (Neukirch an der Thur, Switzerland). In both cases, the entire above-ground material had been harvested from pure stands and sun-dried. During pelleting, the material was heated to max. 60 °C for a short time. The cows were always kept together, either in an open-space barn or on pasture. After each milking (05.00 h and 16.30 h), all cows were fixed in headlocks for 30 to 45 min where they individually received 1 kg portions (as fed) of the respective experimental pellets. Cows grazed on a natural grass-rich pasture from about 07.00 h to 16.00 h. During night in the barn, they were provided with a cut grass-clover mixture consisting of ryegrass (*Lolium multiflorum*) and red clover (*Trifolium pratense*) as well as grass hay from a grass-rich natural meadow. Limited grass growth on pasture due to dry weather conditions forced the farmer to keep the cows in barn during the last two days in week 3 and for the first five days in week 4. During this time, the intake from pasture was replaced by a higher offer of the grass-clover mixture. Sampling was not affected, as it was performed in weeks 0 (baseline), 3 and 5, i.e. before and after these days of complete indoor stay.

The 3 × 10 cows were allocated to the treatment in a completely randomised design. Group averages were balanced for a number of traits. Accordingly, cows of CON, SST and SLT had an initial milk yield (arithmetic mean ± one standard deviation) of 20.3 ± 4.3, 18.8 ± 5.4 and 19.9 ± 5.3 kg/day, respectively. The corresponding initial averages for milk urea concentration, days in milk and number of lactations (arithmetic mean ± one standard deviation) were 44.7 ± 2.3, 44.5 ± 3.7 and 45.2 ± 3.6 mg/dL, 167 ± 76, 160 ± 66 and 159 ± 66 days, and 3.2 ± 2.3, 3.0 ± 1.7 and 3.0 ± 2.1 lactations. Finally, data from one SLT cow had to be excluded from the experiment due to nervous behaviour.

2.2. Data and sample collection

Samples of each forage were taken 11 times, evenly distributed across the experiment. For this purpose, pasture forage was manually cut from the actual pasture 5 cm above ground in five plots of 0.25 m^2; these cuts were always taken from paddocks immediately before they were opened for grazing. Samples of the fresh grass-clover mixtures and grass hay fed in barn were taken at five points across the barn trough at each sampling event. Samples of the ryegrass and sainfoin pellets were

collected two to three times per week and then pooled to one sample per week. The samples of fresh plants were dried at 40 °C during 48 h. Afterwards, all feed samples were milled through a 0.5 mm sieve (Retsch SK 100, Retsch®, Haan, Germany).

To record eating and rumination behaviour, sensor halters (RumiWatch®, Itin + Hoch GmbH, Liestal, Switzerland; Rombach et al., 2018) were mounted on to all cows in first and second lactation during the sampling weeks (0, 3 and 5). In week 0, it was possible to evaluate six halters each from groups CON and SST, but from group SLT only two because of technical problems. In weeks 4 and 6, groups SST and C each wore five halters, group SLT six. Sensor data were converted to eating and rumination times (min/d and min/h) with the Rumiwatch converter V0.7.3.2 (Rombach et al., 2018). The records collected during the respective sampling weeks were evaluated for the time between 05.00 h on Tuesday until 05.00 h on Friday (i.e., 72 h). Values of eating time, rumination time and number of activity changes were calculated for entire days (min/d or n/d) and for three smaller segments, namely from 05.00 h to 13.00 h, from 13.00 h to 21.00 h and from 21.00 h to 05.00 h (min/h or n/h).

Individual milk yields were quantified and milk samples were taken on Tuesday evening, Wednesday morning, Thursday evening and Friday morning of each sampling week. Milk samples were conserved with Bronopol® and pooled to one sample per animal and week by mixing the samples proportionately to milk amounts obtained at the respective milking events. Along with milk sampling, faeces and urine spot samples were taken. Faeces samples were taken rectally from each cow and stored at 4 °C. These samples were pooled at similar portions to one sample per cow and week at the end of each sampling week. Half of the pool sample was dried at 40 °C for 48 h and then milled through a 0.5 mm sieve for laboratory analysis. The other half was stored at -20 °C for later sieve washing. Urine excreted spontaneously by each cow was collected during pellet feeding in the headlocks after milking and subsequently acidified to pH 2-3 with sulphuric acid (20%; *v/v*). An amount of 50 ml of each urine sample was filtered (Whatman™ filter paper 1, GE Healthcare, Buckinghamshire, Great Britain), and 10 ml of the filtered urine was diluted with 40 ml distilled water (1:5, *v/v*) and vortexed. Three 15-ml aliquots were then stored at -20 °C.

2.3. Laboratory analysis

Dry matter (DM) concentrations of all fresh feed and faeces samples were determined by drying them at 40 °C for 48 h. Concentrations of total ash, CP and fibre fractions (neutral and acid detergent fibre (NDF and ADF)) as well as crude fibre (all ash-corrected) were determined by near infrared spectroscopy (NIRS, NIRFlex N-500, Büchi, Flawil, Switzerland). Calibration of the NIRS device had been carried out with results of wet chemically analysed samples for both 180 forage samples from grass-herb based swards and 45 faeces samples. Samples for calibration for faeces analysis were taken during the experiment in the present study and additionally from four other farms feeding diets of different composition. In addition, ash-free acid detergent lignin (ADL) was determined in all forage and faeces samples by means of sulphuric acid (72%, v/v) using Fibertherm FT 12 (C. Gerhardt GmbH & Co. KG, Königswinter, Germany). Phenol analysis was performed in all feeds and pooled per week according to the laboratory manual of Makkar (2003), with slight modifications. Briefly, extracts were prepared with 70% aqueous acetone (*v/v*), which were subsequently filtered (Cameo™ syringe filter™, non-sterile, pore size 1.2 μm). Phenols measured included total extractable phenols and non-tannin phenols using tannic acid as a standard. In addition, condensed tannins (CT) were quantified by butanol-HCl assay (Makkar, 2003) with leucocyanidin as standard. Total tannins were calculated as total extractable phenols minus non-tannin phenols.

In the thawed faeces samples, particle size distribution was quantified according to Leiber et al. (2015) using four stapled sieves with different mesh sizes (4, 2, 1 and 0.3 mm diameter). Residues were dried at 105 °C for 12 h, subsequently weighed, and proportions of the fractions were calculated.

Milk samples were analysed at the Swiss routine milk analysis laboratory (Suisselab AG, Zollikofen, Switzerland) for fat, protein, lactose and urea by Fourier transform infrared spectroscopy (MilkoScan FT 6000, Foss Electric, Hillerød, Denmark).

The purine derivatives (PD) allantoin and uric acid, and creatinine in urine were analysed with high-performance reversed-phase liquid chromatography (Dickhoefer et al., 2015). Allantoin and uric acid were considered as total PD, because hypoxanthine and xanthine are almost absent in cattle urine (Verbic et al., 1989). Total urinary N was analysed by Kjehldahl method (AOAC 991.20; AOAC, 2000) applying the Gerhardt KT20 (C. Gerhardt, Königswinter, Germany) for digestion and a Kjeldahl apparatus (Büchi B324, Büchi Labortechnik, Essen, Germany) for distillation.

2.4. Calculations and statistical analysis

Net energy for lactation (NEL) and absorbable protein at the duodenum based on rumen-undegradable nitrogen compounds plus microbial protein either from fermentable energy (APDE) or from rumen-degradable nitrogen compounds (APDN) were calculated according to the regressions of Agroscope 2020 using concentrations of DM, total ash, CP and crude fibre as measured in the feeds.

Total urine volumes could not be determined. However, daily urinary creatinine excretion is considered to be constant as it reflects maintenance muscle protein turnover (Chizzotti et al., 2008). Therefore, urine parameters can be related to it, to account for inter-animal and day-to-day variations in urine volume.

The PD:C ratio is an indicator of microbial protein synthesis in rumen (Tas and Susenbeth, 2007; Chizzotti et al., 2008):

(4) PD:C ratio = (allantoin [mmol/l] + uric acid [mmol/l]) / creatinine [mmol/l].

To determine the PD:creatinine index (Chen and Ørskov 2004), the individual body weight (BW) of the experimental cows was estimated before and after the experiment with a weighing tape (Vieh- und Schweinemessband 250 cm, Hoechstmass Balzer, Sulzbach, Germany) and the average BW was included in the following formula.

(5) PD:C-index = (allantoin [mmol/l] + uric acid [mmol/l]) / creatinine [mmol/l] × BW [$kg^{0.75}$].

Next, the PD:N ratio was calculated as an indicator of the efficiency of use of ingested N for microbial protein synthesis in the rumen (Tas and Susenbeth, 2007) as:

(6) PD:N ratio = (allantoin [mmol/l] + uric acid [mmol/l]) / N [g/l].

Finally, the N:creatinine ratio (N:C), an indicator of N excretion in urine (Chizzotti et al., 2008), was computed as:

(7) N:C ratio = N [g/l] / creatinine [mmol/l].

Data were analysed with SPSS® statistical software version 24, using a general linear model. Dietary treatment, sampling week (i.e., 3 and 5) and their interaction were used as fixed factors, while data from week 0 were used for weighted least squares corrections on animal level.

The model used, where week-0 data were considered as covariate (COV), was:

$$Y_{i,j} = \mu + COV + \alpha \times treatment_i + \beta \times week_j + \gamma \times treatment_i \times week_j + \varepsilon_{i,j}$$

Least squares means were calculated and presented in the tables. Multiple comparisons among the least squares means for each treatment across both weeks were performed with Tukey's procedure, considering $P < 0.05$ as significant and $P < 0.10$ as tendency.

3. Results

3.1. Diet composition

The chemical composition of the main forages changed during the course of the experiment (Table 1), which was also due to variable

Table 1
Chemical composition of the basal diet components (g/kg dry matter ± standard deviation) in the sampling weeks (mean of two samples with three replicates per sample).

	Fresh grass-clover mixture fed in barn			Grass hay fed in barn			Pasture forage		
Week	0	3	5	0	3	5	0	3	5
Estimated dietary proportion (g/kg)[1]	389	385	385	278	274	163	333	219	329
Analysed variables									
Dry matter (g/kg fresh matter)	174 ± 0.0	199 ± 1.8	200 ± 18.6	923 ± 0.2	785 ± 39.8	874 ± 55.4	233 ± 60.7	484 ± 12.1	NA
Ash	105 ± 3.1	129 ± 9.3	138 ± 7.8	98 ± 6.7	97 ± 5.9	115 ± 2.5	82 ± 4.7	82 ± 6.3	118 ± 2.6
Crude protein	210 ± 1.5	202 ± 9.7	173± 5.4	95 ± 9.5	92 ± 2.7	107 ± 2.4	161 ± 13.5	122 ± 5.9	248 ± 1.5
Neutral detergent fibre	266 ± 12.0	264 ± 40.5	284 ± 9.4	489 ± 18.5	479 ± 25.8	520 ± 29.1	498 ± 88.2	486 ± 69.6	213 ± 2.8
Acid detergent fibre	175 ± 9.4	180 ± 16.3	147 ± 12.1	327 ± 9.7	323 ± 16.3	386 ± 17.9	230 ± 29.0	211 ± 30.4	73 ± 2.5
Acid detergent lignin[2]	42.0 ± 2.83	31.6 ± 7.03	36.9 ± 2.80	40.4 ± 2.68	44.0 ± 0.09	52.4 ± 1.59	33.0 ± 1.50	46.8 ± 0.08	27.9 ± 2.82
Crude fibre	201 ± 5.5	225 ± 13.8	222 ± 5.6	288 ± 3.2	289 ± 8.8	324 ± 12.8	269 ± 17.9	276 ± 19.3	157 ± 1.2
Total extractable phenols[2,3]	28.4 ± 0.58	17.1 ± 1.17	24.9 ± 1.77	10.8 ± 0.89	16.2 ± 7.03	10.7 ± 0.26	25.0 ± 2.94	17.6 ± 0.99	22.1 ± 1.50
Non-tannin phenols[2,3]	21.8 ± 1.21	16.8 ± 0.01	21.7 ± 1.98	10.4 ± 0.10	10.2 ± 0.41	9.8 ± 0.60	21.2 ± 0.98	17.2 ± 0.53	20.9 ± 1.07
Total tannins[3,4]	6.61 ± 1.566	0.35 ± 0.345	3.19 ± 2.372	0.52 ± 0.346	5.99 ± 1.218	0.88 ± 0.192	3.80 ± 1.965	0.34 ± 0.000	1.21 ± 0.832
Calculated variables									
NEL (MJ/kg dry matter)	5.53 ± 0.047	5.25 ± 0.150	5.20 ± 0.058	4.35 ± 0.050	4.26 ± 0.141	3.68 ± 0.254	5.08 ± 0.249	4.72 ± 0.219	5.43 ± 0.047
APDE (g/kg dry matter)	98.3 ± 0.47	93.7 ± 2.69	89.3 ± 1.11	69.3 ± 2.36	67.5 ± 1.89	65.3 ± 3.09	83.0 ± 3.78	78.5 ± 2.87	102.7 ± 0.47
APDN (g/kg dry matter)	134.8 ± 1.21	129.7 ± 6.05	111.2 ± 3.72	59.7 ± 6.05	58.0 ± 1.83	67.8 ± 1.46	103.0 ± 8.67	77.7 ± 3.82	158.7 ± 0.94

APDE/APDN, absorbable protein at the duodenum consisting of rumen-undegradable protein and microbial protein from fermentable energy/ rumen-degradable protein; NA = not available; NEL, net energy for lactation.
1 Estimates based on weighings of the barn feeds and an assumed total DM intake of 18 kg/day/cow, including the offered pellets (1.8 kg DM /day) in weeks 3 and 5.
2 Samples pooled per week before analysis.
3 Tannic acid equivalents.
4 Difference between the overall mean of total extractable phenols and non-tannin phenols.

weather conditions. The latter also influenced the daily time cows were turned out to pasture and, thus, estimated DM intake from pasture (lower in week 3 compared to weeks 0 and 5). The CP concentrations of the forage fed in the barn and on pasture developed differently. During the experiment, the CP concentration decreased from week 0 to 5 in the grass-clover mixture, whereas it increased in pasture towards the end of the trial. In week 5, the concentrations of NDF and ADF in pasture herbage were very low. In week 3, the ratios of APDN to APDE were 1.12 and 1.15 for CON and for the sainfoin groups, respectively. The corresponding ratios were 1.31 and 1.32 in week 5. The concentrations of total tannins in the forages varied with time as well, but were generally very low, and no CT could be detected. The concentrations of the non-tannin phenols were clearly higher than those of the tannins. The concentrations of total extractable phenols, non-tannin phenols and total tannins were much higher in the sainfoin pellets compared to the ryegrass pellets (Table 2), and CT could be detected only in the sainfoin pellets. About 80% of the CON pellets offered were consumed, and the sainfoin pellets were almost completely eaten in group SST and to around 96% in group SLT (Table 3).

3.2. Eating and rumination behaviour

Eating time per day and per hour did not differ among groups (Table 3, overall treatment means across both weeks not shown in table). Regarding the smaller time intervals, SST resulted in a shorter ($P < 0.10$) eating and longer rumination time ($P < 0.05$) between 05.00 h and 13.00 h compared to CON, but no other group differences occurred. The number of activity changes remained unaffected by the treatments.

3.3. Milk yield and composition

Milk yield did not differ among groups (Table 4). However, milk fat and milk protein yields were greater ($P < 0.05$) with SST than with CON, with SLT being intermediate. This was the effect of the combination of numerical differences in both milk yield and milk fat/protein concentrations. Treatment SST led to a lower ($P < 0.05$) lactose concentration in milk compared to SLT, whereas the values of both groups did not differ to those of CON. Milk urea concentration decreased ($P < 0.001$) from week 3 to 5, and there was a trend ($P < 0.10$) towards lower values with both sainfoin treatments, irrespective of the sampling week, compared to CON. However, milk urea yield did not differ among groups. The BW of cows did not differ among groups.

Table 2
Chemical composition (g/kg dry matter) of sainfoin and ryegrass pellets in the sampling weeks (mean of two samples with three replicates per sample ± standard deviation)

	Sainfoin	Ryegrass
Analysed variables		
Dry matter (g/kg fresh weight)	928 ± 4.3	946 ± 5.2
Total ash	111± 3.6	67 ± 2.8
Crude protein	128 ± 4.4	45 ± 2.3
Neutral detergent fibre	444 ± 4.9	697 ± 1.5
Acid detergent fibre	409 ± 4.4	484 ± 2.7
Acid detergent lignin[1]	74.9 ± 10.46	55.8 ± 7.30
Crude fibre	305 ± 6.1	382 ± 7.3
Total extractable phenols[1,2]	78.0 ± 1.53	6.4 ± 0.22
Non-tannin phenols[1,2]	29.4 ± 0.33	5.9 ± 0.11
Total tannins[1,3]	48.6 ± 1.89	0.5 ± 0.39
Condensed tannins[1,4]	37.8 ± 0.71	ND
Calculated variables		
NEL (MJ/kg dry matter)	4.67 ± 0.109	3.56 ± 0.143
APDE (g/kg dry matter)	79.7 ± 1.83	45.7 ± 1.42
APDN (g/kg dry matter)	81.4 ± 3.04	13.7 ± 2.28

APDE/ APDN, absorbable protein at the duodenum consisting of rumen-undegradable protein and microbial protein from fermentable energy/ rumen-degradable protein; ND, not detected; NEL, net energy for lactation.
1 Samples pooled per week before analysis.
2 Tannic acid equivalents.
3 Difference between the overall mean of total extractable phenols and non-tannin phenols.
4 Leucocyanidin equivalents.

Table 3
Effect of short-term versus long-term sainfoin supplementation on eating and rumination time as well as number of activity changes.[1]

Treatment group (G)	CON[2]			SST[2]			SLT[2]				*P*-values		
Sampling week (W)	3	5		3	5		3	5		SEM	G	W	G × W
Pellet intake (% of offered)	80.0	78.6	B	100	98.6	A	93.5	98.4	A	2.10	<0.001	0.855	0.732
Eating time													
min/day	524	609		510	573		516	603		11.5	0.561	0.003	0.873
05.00-13.00 h (min/h)	28.0	31.3	A	24.5	27.8	B	28.6	28.4	A	0.64	0.047	0.112	0.551
13.00-21.00 h (min/h)	29.7	32.6		30.6	31.3		29.5	33.6		0.84	0.965	0.143	0.687
21.00-05.00 h (min/h)	8.6	12.3		8.6	12.4		6.5	13.8		0.47	0.957	<0.001	0.328
Rumination time													
min/day	494	415		530	448		505	457		10.4	0.262	0.003	0.819
05.00-13.00 h (min/h)	18.6	13.7	B	22.9	17.7	A	17.9	17.6	AB	0.56	0.005	0.006	0.236
13.00-21.00 h (min/h)	13.1	11.9		13.1	12.8		17.4	11.1		0.52	0.465	0.024	0.119
21.00-05.00 h (min/h)	29.8	26.0		29.9	25.6		28.9	29.0		0.58	0.740	0.032	0.356
Activity changes													
n/day	178	150		155	141		144	144		5.2	0.219	0.200	0.555
05.00-13.00 h (*n*/h)	7.8	6.5		7.4	5.9		7.3	5.8		0.30	0.584	0.031	0.987
13.00-21.00 h (*n*/h)	7.7	6.1		6.6	6.4		6.7	7.0		0.25	0.711	0.343	0.261
21.00-05.00 h (*n*/h)	6.6	5.9		5.6	5.5		3.9	5.3		0.33	0.160	0.768	0.482

1Mean of six (SLT) or five (CON and SST) measurements per group with recording time of 72 h.
2 CON, control (ryegrass pellets); SST, sainfoin pellets short-term; SLT sainfoin pellets long-term.
A,B Treatments within a variable marked with different capital letters are significantly different from each other across the experiment ($P < 0.05$).

Table 4
Effect of short-term versus long-term sainfoin supplementation on milk yield and composition.[1]

Treatment group (G)	CON[2]			SST[2]			SLT[2]				*P*-values		
Sampling week (W)	3	5		3	5		3	5		SEM	G	W	G × W
Daily yield													
Total milk (kg/cow and day)	17.3	16.8		19.1	19.0		18.9	18.1		0.518	0.291	0.646	0.955
Fat (g/cow and day)	672	649	B	778	769	A	752	738	AB	0.015	0.012	0.616	0.982
Protein (g/cow and day)	590	596	B	653	681	A	621	639	AB	0.012	0.047	0.468	0.935
Urea (g/cow and day)	5.57	4.73		5.83	4.94		5.96	4.84		0.174	0.808	0.009	0.945
Composition													
Fat (g/100 g milk)	4.13	4.19		4.33	4.38		4.20	4.29		0.084	0.638	0.684	0.993
Protein (g/100 g milk)	3.58	3.74		3.69	3.88		3.52	3.75		0.063	0.564	0.133	0.971
Lactose (g/100 g milk)	4.60	4.62	AB	4.53	4.57	B	4.69	4.69	A	0.016	0.007	0.591	0.896
Urea (mg/dL milk)	31.6	27.4		30.2	25.6		30.2	25.9		0.332	0.099	<0.001	0.974

1 All parameters are means of nine (SLT) or ten samples (CON and SST), each pooled of four samples per cow and week.
2 CON, control (ryegrass pellets); SST, sainfoin pellets short-term; SLT sainfoin pellets long-term.
A,B Treatments within a variable marked with different capital letters are significantly different from each other across the experiment ($P < 0.05$).

3.4. Faeces-related variables

The faecal CP concentration was higher ($P < 0.05$) in both sainfoin groups compared to CON, and the NDF concentrations were lower with SLT compared to CON ($P < 0.05$; Table 5). The proportion of total particles > 0.3 mm in faeces DM was lower ($P < 0.05$) in both sainfoin groups compared to CON, which was mainly caused by differences in the fraction > 0.3 to 1 mm. The proportion of the particles > 4 mm showed a tendency ($P < 0.10$) to be lower in SST compared to CON. In this fraction, an interaction of treatment and week ($P < 0.05$) was also found, whereby the proportion decreased ($P < 0.05$) from week 3 to week 5 in SST and CON, but increased ($P < 0.05$) with advancing experimental

Table 5
Effect of short-term versus long-term sainfoin supplementation on composition of faeces, and particle size distribution in the faeces.

Treatment group (G)	CON[3]			SST[3]			SLT[3]				*P*-values		
Sampling week (W)	3	5		3	5		3	5		SEM	G	W	G × W
Faeces composition (g/kg dry matter)[1]													
Crude protein	141	149	B	147	157	A	142	161	A	0.1	0.006	<0.001	0.076
Neutral detergent fibre	424	422	A	412	406	AB	415	398	B	0.3	0.021	0.108	0.491
Acid detergent fibre	459	456		457	452		468	444		0.2	0.794	0.015	0.116
Acid detergent lignin	177	185		174	179		176	177		0.1	0.058	0.005	0.199
Particles (g/100 g dry matter)[2]													
∑ particles > 0.3 mm	50.2	49.3	A	44.8	44.8	B	43.9	44.8	B	0.82	0.015	0.990	0.900
> 0.3 to 1 mm	36.0	33.1	A	29.7	30.8	B	30.2	31.4	AB	0.71	0.026	0.880	0.407
> 1 to 2 mm	8.91	10.16		8.07	9.45		11.1	10.9		0.47	0.128	0.393	0.723
> 2 to 4 mm	3.85	3.37		3.64	3.60		2.79	3.28		0.133	0.145	0.970	0.348
> 4 mm	3.61	1.85		2.38	1.51		2.35	2.40		0.134	0.058	0.002	0.020

1Mean of nine (SLT) or ten samples (CON and SST) with three values per sample (each pooled of four samples per cow and week).
2 Means per group, made of four samples per cow and week.
3 CON, control (ryegrass pellets); SST, sainfoin pellets short-term; SLT sainfoin pellets long-term.
A,B Treatments within a variable marked with different capital letters are significantly different from each other across the experiment ($P < 0.05$).

Table 6
Effect of short-term versus long-term sainfoin supplementation on urine variables.[1]

Treatment group (G)	CON[2]			SST[2]			SLT[2]				*P*-values		
Sampling week (W)	3	5		3	5		3	5		SEM	G	W	G × W
Total N (g/L)	6.25	4.57	A	4.89	4.59	B	6.18	5.41	A	0.091	< 0.001	<0.001	0.010
Allantoin (mmol/L)	11.16	11.04	AB	9.55	10.81	B	11.65	11.85	A	0.190	0.005	0.243	0.304
Uric acid (mmol/L)	1.62	1.66		1.38	1.69		1.72	1.76		0.044	0.192	0.146	0.348
Creatinine (mmol/L)	4.10	3.84	A	3.28	3.60	B	3.89	3.93	AB	0.087	0.029	0.849	0.381
PD:C ratio[3]	3.16	3.35		3.46	3.65		3.53	3.53		0.061	0.090	0.307	0.765
PD:C index[3,4]	397	423		449	475		442	441		8.9	0.065	0.355	0.773
PD:N ratio[3] (mmol/g)	2.05	2.83		2.26	2.76		2.16	2.50		0.037	0.069	<0.001	0.023
N:C ratio[3] (g/mmol)	1.57	1.21		1.54	1.31		1.67	1.41		0.028	0.080	<0.001	0.579

1All parameter means of nine (SLT) or ten samples (CON and SST) with three values per sample (each pooled of four samples per cow and week).
2 CON, control (ryegrass pellets); SST, sainfoin pellets short-term; SLT sainfoin pellets long-term.
3 PD, purine derivatives; N, nitrogen; C, creatinine.
4Adjusted by body weight of the cows.
A,B Treatments within a variable marked with different capital letters are significantly different from each other across the experiment ($P < 0.05$).

duration in SLT.

3.5. Urine-related variables

Urinary PD:C ratio and index (i.e., adjusted for BW) tended ($P < 0.10$) to be higher with SST compared to CON (Table 6). Group SLT tended ($P < 0.10$) to have a higher ratio of N:C than CON. There was a treatment and week interaction ($P < 0.05$) in the PD:N ratio, where the increase from week 3 to week 5 was greater with CON than with both sainfoin groups. Moreover, SST tended ($P < 0.10$) to have a higher PD:N ratio than SLT, both with no difference to CON.

4. Discussion

4.1. On-farm experimental approach

The main goal of the present study was to confirm whether feasible levels of sainfoin supplementation affect the protein metabolism of dairy cows under on-farm conditions with easily measurable indicators. The real conditions of a commercial dairy farm differ from the controlled, constant and technical environment of a research station. The results of such on-farm experiments are restricted to the specific and partly unpredicted conditions under which they are generated. They clearly have the character of a case study. However, this is also true for pasture-based experiments on research stations when natural grasslands with high heterogeneity are investigated (Leiber et al., 2019), reflecting the extremely high variability in forage composition in and between pasture-based systems. This general problem cannot be overcome only by standardised station experiments, but rather by large numbers of similar experiments or data collections under varying on-farm conditions (Richter et al., 2009; Chobtang et al., 2016; Leiber et al., 2017). There is a clear trade-off between low numbers of farms with high quality of variables and vice-versa. The approach of the current study was one of the extremes: only one farm with a maximum of measured and analysed variables – thus an in-depth described case study. Such a case cannot be generalised, but it can function as a counterpart or addition to on-station results, whenever the question of practical applicability is debated. A further constraint of on-farm experiments is that methods of data and sample acquisition, which are invasive or require fixing the animals in their stand, such as for blood and rumen fluid sampling or complete excreta collection, can seldom be applied to privately owned animals. The indicators easiest to obtain and likely most comprehensive are those derived from milk, as information about individual milk yield is available from routine testing and almost always an aliquot of the whole volume milked in 24 h can be recorded. As concerns faeces and urine, only spot sampling is feasible on-farm, meaning that variables derived from these samples can have only proximate character but may still bear complementary information, in particular regarding the pathways of nitrogen metabolism. The chewing sensors used provide further information related to intake and rumination (Kovacs et al., 1997; Rombach et al., 2019). In the present context, they can be informative proxies of animal responses to herbal feed additives. Milk yield and composition aside, all parameters used in this experiment must be considered as proxies with the aim to provide a best possible overall characterisation of the effects of sainfoin supplementation.

4.2. Suitability of basal diet, sainfoin pellets and supplementation schedule

During the experimental period, the basal diet showed, as anticipated, a clear excess of APDN compared to APDE, which indicates that the supply with rumen-degradable protein was too high in relation to the energy available for rumen microbial protein synthesis. Sainfoin as model plant for providing tannins to counteract poor ruminal CP utilisation contained 37 g/kg DM of CT. This concentration was in a range similar to that in other studies (Aufrere et al., 2008; Bouchard et al., 2013; Scharenberg et al., 2008). Per day, each cow in the sainfoin groups received 70 g CT (equivalent to approximately 4 g CT/kg dietary DM when assuming a total intake of 18 kg DM/day). The CT mainly, if not exclusively, came from the sainfoin because CT concentrations in the other feeds were not detected. With this dietary CT level no adverse impact on feed intake, nutrient digestion and animal health is expected (Aerts et al., 1999a; Bouchard et al., 2013; Scharenberg et al., 2009). The daily CT supplementation was at the lower threshold of dietary CT concentrations at which even positive effects can be anticipated (Scharenberg et al., 2009). On the other hand, the quantity was in a realistic range for on-farm use, because a supply of more than 2 kg/day (as fed) of sainfoin per cow does not appear feasible due to the high costs of production.

A true shortcoming in the present study was that the control pellets made from ryegrass contained unexpectedly low crude protein and energy. This doubtlessly affected yields and nitrogen budgets, and limited the conclusions based on comparison with the control. The fact that the control cows had the lowest protein supply therefore has to be considered when drawing conclusions from this experiment. In the present study, continuous and intermittent addition of sainfoin pellets to the diet were compared in order to determine whether effects differ between short-term and long-term application (Aerts et al., 1999b; Khiaosa-ard et al., 2012). This approach was chosen in order to test a low-input vs. a high-input sainfoin strategy. If an intermittent short-term supply were found to be similarly effective as a continuous supply, scarce sainfoin resources could be used more efficiently in this way instead of risking a lack of effect by a continuous but too low supply with sainfoin CT. Indeed, effects of short-term supplementation of sainfoin (SST) were even more pronounced compared to continuous supply (SLT) over three

and up to five weeks. This observation may have been due to an increasing adaptation in ruminal protein metabolism along with continuous supplementation of sainfoin (Matthews et al., 2019). A successful application of sainfoin supplementation appeared to be restricted to periods no longer than a week in the current study. This finding does not generally exclude that long-term sainfoin supplementation is effective as well (Zhang et al., 2019). It can, however, also not be excluded that the main cause for the effect of intermittent supply was the abrupt and repeated change between pellet types. The exact causality underlying the differences between SST and SLT in the current study is limited; nonetheless, the effect may be relevant for application of sainfoin supplementation in farm practice.

4.3. Eating and rumination behaviour, digestibility estimates and urinary indicators

Daily eating and rumination times as observed in our study were similar to those in other on-pasture studies (Rombach et al., 2018; Rombach et al., 2019). Although during the first half of the day rumination time was longer in SST compared to CON, general treatment effects on rumination and eating behaviour were negligible, and an influence of sainfoin supplementation on these variables cannot be concluded.

Indicators of metabolic protein utilisation and excretion in urine can be grouped into urinary N and PD. A part of urinary N also originates from the ammonia formed during CP degradation by rumen microbes, which is absorbed, detoxified in the liver and excreted mainly as urea. Urinary N thus depends on the composition of the diet, mainly on its N to energy balance (Dijkstra et al., 2013). The PD in urine mainly originate from microbial nucleic acids digested and absorbed in the small intestine. They are are used to estimate microbial protein synthesis in rumen (Tas and Susenbeth, 2007).

With sainfoin supplementation during short periods, the PD:C ratio and index tended to be higher compared to CON, indicating an increase in rumen microbial growth in the sainfoin groups as compared to CON, which well may be due to the lower protein supply in the latter treatment group. Furthermore, urinary nitrogen was lower with SST compared to SLT and CON. Together with a higher protein yield, this may indicate that the intermittent supplementation of sainfoin resulted in improved N utilisation compared to CON, with a clearer effect than a continuous supply.

4.4. Effects on milk yield and composition

Associated with a higher synthesis of microbial protein and a lower metabolic load of ammonia absorbed from the rumen, supplementing sainfoin as a tannin-rich herb was expected to increase the yield of milk protein and decrease that of milk urea (Totty et al., 2013). In the present study, milk protein yield did indeed change as anticipated, but part of this effect was likely due to the poor quality of the supplement in CON. However, in both sampling weeks the effect of SST was stronger than that of SLT, suggesting that particularly the intermittent supply might be of advantage. Milk urea yield was not affected by dietary treatment, likely due to the comparatively low level of tannins in sainfoin (Scharenberg et al., 2009). Hence, the chosen levels of sainfoin supplementation were too low to induce pronounced effects on production and protein metabolism, indicating that there is a clear trade-off between production costs for sainfoin supplements and their effectiveness in practice. This is in line with similar findings regarding hydrolysable tannin supplements (Kapp-Bitter et al., 2020). Thus, practically applicable solutions to manipulate nitrogen efficiency in grazing cows continue to be a challenge.

5. Conclusions

An experiment was conducted under practical farming conditions with a feasible amount of sainfoin in diets for dairy cows fed high-protein autumn herbage. With interrupted supplementation of sainfoin in two five-day periods, the milk protein yield slightly increased and the urinary PD indicated an improved microbial growth rate compared to longer-lasting sainfoin feeding. Results suggest that sainfoin might affect cattle protein conversion more efficiently when applied intermittently for short periods. However, the effects were generally small, and there is a clear trade-off between applicably low levels of sainfoin supplementation and the expected benefits. The limitations regarding the measured parameters of the experiment, which were due to its practice-oriented on-farm situation, do not contradict this conclusion. A shortcoming of the study consisted in the low CP content of the control pellets. This, however, did not impair the direct comparison of short-term to long-term sainfoin supply.

Declaration of Competing Interest

None.

Acknowledgments

We are grateful to Alois Huber, Wildegg, for providing his Swiss Fleckvieh herd for this experiment and to the laboratory staff of FiBL and the University of Hohenheim for supporting the analyses. The project was funded by the Swiss National Science Foundation (project no. 31003 A_166425).

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