Journal of Animal and Feed Sciences, 29, 2020, 97–104 https://doi.org/10.22358/jafs/121053/2020
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Graded supplementation of chestnut tannins to dairy cows fed protein-rich spring pasture: effects on indicators of protein utilization

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KEY WORDS: cattle, protein efficiency, low-input, hydrolysable tannin, field experiment, chewing sensor

Received: 31 January 2020
Revised: 13 March 2020
Accepted: 24 April 2020

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ABSTRACT. An on-farm experiment was conducted in order to evaluate effects of graded supplementation with chestnut tannin extract to cows in situations of excessive dietary protein supply on a low-input organic dairy farm. Respectively 10 Swiss Fleckvieh cows received twice per day 1 kg of experimental pellets containing either 0, 50 or 100 g/kg of chestnut extract (targeted at approximately 0, 5 and 10 g extract/kg of total dietary dry matter). Experimental feeding lasted for 21 days. Measurements and collection of milk, faeces and urine spot samples were performed in weeks 0 (baseline), 1 and 3. All cows were kept in one herd on pasture; fresh grass and grass hay were provided in the barn during night. Milk yield was recorded and cows wore sensor halters for recording chewing activity. In urine, total nitrogen and purine derivatives were measured; faeces were analyzed for protein, fibre and particle fractions; in milk, solid concentrations were determined. The data was analyzed with a general linear model. Cows did not show differences in general eating and rumination behaviour, but needed time to accept the tannin-containing pellets. Milk yield and composition were not affected by treatment, except for lactose content. No relevant differences between treatments were found for urinary and faecal parameters. In conclusion, although technically easy to supplement, pellets containing chestnut tannin extract were not readily accepted by the cows and effects on protein digestion and metabolism were not found. Successful on-farm application of chestnut extract in order to improve nitrogen efficiency therefore seems questionable.

Introduction

Against the background of the increasing ecological pressure due to land-use for feed concentrate production (Wilkinson, 2011; Schader et al., 2015), low or zero feeding of concentrates to ruminants is one option in discussion (Ertl et al., 2015; Leiber et al., 2017). One of the associated challenges is to improve the conversion of feed nitrogen (N) into milk N when nutritional imbalances in the forage cannot be counterbalanced with concentrates. In low-input systems protein excess in pasture grass often occurs during spring and autumn (Pacheco and Waghorn, 2008). This excess protein is degraded to ammonia in the rumen and is at least partly not used by the rumen microbes. Ammonia has to be metabolized in the liver under energy consumption (Parker et al., 1995) and is excreted largely as

easily volatile urea *via* urine. Hence, protein excess in cattle diets is a burden for the cow's metabolism and for the environment.

A possible solution could be to feed tannin-rich plants and profit from potentially positive effects on the protein metabolism of ruminants (Mueller-Harvey, 2006). Tannins may form complexes with dietary proteins in the rumen and thereby reduce proteolysis; the protein could be released in the abomasum (McSweeney et al., 2001). One promising tannin-containing feed supplement is the extract from the chestnut tree (*Castanea sativa* Miller), consisting primarily of hydrolysable tannins (Mueller-Harvey, 2006). *In vitro*, chestnut extract was found to reduce ruminal protein degradation without influencing microbial protein synthesis (Wischer et al., 2013). Chestnut tannin extract therefore may reduce the metabolic load from excessive ammonia *in vivo* and either increase the supply with protein released from the tannin-protein complex or enhance its excretion as quite stable faecal compounds (Mueller-Harvey, 2006). Supplementing chestnut extract was found to reduce N losses (Śliwiński et al., 2004) and methane emissions (Duval et al., 2016) from the manure of dairy cows. Concomitantly, milk yield was not (Duval et al., 2016) or even positively affected (Ali et al., 2017), and no toxic effects or decline in feed intake was reported (Śliwiński et al., 2004; Duval et al., 2016). As the protein excess in grass is usually timely limited, chestnut extract could be supplemented on a farm with reasonable costs. It is, however, unknown, if the effects of such extracts are substantial and can be detected and quantified also under practice conditions of a commercial farm.

The aim of this study was, therefore, to investigate if adding graded levels of chestnut tannin extract results in an improved protein digestion and metabolism under practical conditions with excessive N supply from spring pasture. For this purpose, dehydrated grass pellets were produced as a vehicle for chestnut extract supplementation. Data and samples related to chewing behaviour, milk performance, and faeces and urine composition were obtained. The hypothesis was that the supplemented chestnut extract would lower ruminal protein degradability and eventually improve protein efficiency by better utilization in the duodenum. Under commercial on-farm conditions, this should be indicated by reduced milk urea concentrations and eventually either higher milk protein yields or decreased apparent protein digestibility, or both. Purine derivatives in urine should be indicative for changes in ruminal microbial protein synthesis.

Material and methods

Experimental design and protocol

This experiment took place from April until May 2017 on an organic dairy farm and was approved by the cantonal veterinary office of Aargau, Switzerland (approval no. AG75689). A pre-treatment sampling week (week 0) served to collect baseline data. For the following 21 days (weeks 1–3), 30 lactating Swiss Fleckvieh cows were randomly assigned to three groups of ten animals each and allocated to treatments with pellets containing 0, 50 or 100 g/kg of chestnut tannin extract (TAN0, TAN50 or TAN100); targeted at approximately 0, 5 or 10 g extract/kg of total dietary dry matter (DM) based on supplementation of 2 kg pellets/day and an estimated total daily feed intake of 20 kg DM for all cows (Leiber et al., 2015a). Data and sample collection were carried out in weeks 0, 1 and 3. The initial average milk yield before the experiment for the three groups of ten animals each (± standard deviation) was 25.4 ± 5.2, 25.3 ± 4.9 and 25.5 ± 5.4 kg/day, respectively. The corresponding values for days in milk were 131 ± 58, 118 ± 54 and 116 ± 75; cows had 3.6 ± 1.7, 3.6 ± 1.9 and 3.4 ± 2.0 lactations, and milk urea content was 19.5 ± 5.0, 20.7 ± 5.0 and 19.4 ± 4.6 mg/dl. Cows were kept together in one herd in an open-space barn and went to pasture (natural grass-rich pasture on a nutrient-rich ley soil) during the day between milking times from 5:00 to 16:30. During night, a freshly cut grass-clover mixture consisting of *Lolium multiflorum* and red clover (*Trifolium pratense*) was offered in barn *ad libitum*. Because of sudden frost and snowfall, duration of pasture access had to be halved in the last six days of the experiment, with the cows receiving more of the fresh grass-clover mixture and 3 kg DM/day of extra meadow hay (*L. perenne*) per cow in barn for compensation.

After morning and evening milking, cows were fixed in headlocks for 30 min and were individually fed 1 kg of the experimental pellets (totally 2 kg/cow per day) produced by UFAG (Herzogenbuchsee, Switzerland). Pellets consisted of dehydrated *L. perenne*, 100 g/kg of molasses and either 0, 50 or 100 g/kg of chestnut tannin extract (as fed). The rather low levels of supplementation had been chosen in order to avoid detrimental effects (Jayanegara et al., 2011; Henke et al., 2017) and to be economically feasible under practice conditions. The tannin extract (powder) had been prepared from *Castanea sativa* Miller (Farmatan 75®, Tanin Sevnica, Sevnica, Slovenia), and contained (g/100 g of extract): pure tannins (75, including

castalagin, vescalagin, castalin and vesaclin) as well as sugar (16), water (7) and crude ash (2), according to the producer.

Data and sample collection

Grazed and mowed grasses were sampled twice per week during the experiment; the hay was sampled twice in the last week, as it was only fed then. For each sampling, samples of the pasture forage were taken from five randomly distributed places, each over 1 square meter, and cut 5 cm above the ground. Samples of the grass hay and fresh grass-clover mixture fed in the barn were taken from five places distributed over the whole length of the feeding bank. Pellet samples were taken three times. Forage samples were dried at 40 °C for 48 h. All samples were milled through a 0.5-mm-sieve (Retsch SK 100, Retsch®, Haan, Germany).

In all sampling weeks, pressure sensor halters (RumiWatch®, Itin + Hoch GmbH, Liestal, Switzerland) were mounted on six cows per group for 5 days, except for TAN100, of which seven animals were chosen. Data obtained between 5:00 on Tuesday and 4:59 on Friday of each sampling week were converted to eating and ruminating time and number of activity changes (Rumiwatch converter V0.7.3.2; Itin + Hoch GmbH, Liestal, Switzerland; Rombach et al., 2018). Based on this, the mean values per day and hour were calculated. Days were also subdivided into diurnal phases (5:00 to 13:00, 13:00 to 21:00 and 21:00 to 5:00) in order to analyze more details of behavioural patterns throughout the day.

Samples of milk, faeces and urine were taken on Tuesday and Thursday evenings, and Wednesday and Friday mornings in the sampling weeks. Milk yield was recorded at each milking. The corresponding evening and morning milk samples were mixed proportionaly to milk amounts per cow and conserved with Bronopol®. Individual body weight was estimated in weeks 0 and 3 of the experiment with a weighing tape (Vieh- und Schweinemessband 250 cm, Hoechstmass Balzer GmbH, Sulzbach, Germany). Faeces samples were taken from the rectum of each cow and were stored at 4 °C. On Fridays of each week, samples were pooled at equal proportions to one sample per cow per week. Half of the pooled sample was kept at −20 °C, the other half was dried at 40 °C for 48 h and milled to 0.5-mm-particle size afterwards. Urine was collected individually from cows when spontaneously excreted or after manual stimulation. To avoid pollution, approximately 200 ml of urine were taken three seconds after starting of excretion (Chizzotti et al., 2008). Afterwards, the samples were acidified to pH 2–3 with 20% sulphuric acid (v/v). Afterwards, the urine samples were filtered (Whatman™ filter paper 1), diluted with distilled water (1:5, v/v) and frozen at −20 °C. Before analyzing, the samples were pooled to one sample per cow and week.

Laboratory analysis

With near infrared reflectance (NIR) spectroscopy (NIRFlex N-500, Büchi, Flawil, Switzerland) the concentrations of dry matter (DM), total ash, crude protein and fibre fractions (i.e., neutral and acid detergent fibre, NDF and ADF) in forage and faeces samples were determined. The NIR device was calibrated with 180 forage (from different grass-herb swards) and 45 faeces samples (from five different farms, including samples from the present study), which had been analyzed for proximate compounds with standard methods (Leiber et al., 2015a). The content of ash-free acid detergent lignin (ADL) in forage samples was determined with sulphuric acid (75%, v/v) (Fibretherm FT 12, C. Gerhardt GmbH & Co. KG, Königswinter, Germany). For phenol analysis, the protocols of Makkar (2003) were followed, and concentrations of condensed tannins (CT) were determined with the butanol-HCl-assay. Total extractable phenols (TEP) and non-tannin phenols (NTP) were expressed as tannic acid equivalents, CT were expressed as leucocyanidin equivalents. Total tannins were calculated as TEP minus NTP. The particle size distribution of faeces was determined according to Leiber et al. (2015b) by washing faeces samples sequentially through sieves with mesh sizes of 4.0, 2.0, 1.0 and 0.3 mm diameter and drying the residues for 12 h at 105 °C. Milk samples were analyzed for fat, protein, lactose and urea with a Fourier transform infrared spectroscopy (MilkoScan FT 6000, Foss Electric, Hillerød, Denmark). The total urine N was determined with a Kjeldahl apparatus (Büchi B324, Büchi Labortechnik GmbH, Essen, Germany) after digestion with the Gerhardt KT20 (C. Gerhardt GmbH & Co, Königswinter, Germany). Allantoin, uric acid and creatinine (CR) were determined by high-performance reversed-phase liquid chromatography following Dickhoefer et al. (2015). The sum of allantoin and uric acid was considered to reflect total purine derivatives (PD). The CR excretion *via* urine was considered to be constant relative to the animals' protein and thus body mass (Chizzotti et al., 2008). Hence, CR concentration in urine was used to correct N and PD concentrations for differences in urine volume and dilution rate.

Calculations

Net energy for lactation (NEL) and absorbable protein at the duodenum, based on rumen-undegradable protein plus microbial protein either from fermentable energy (APDE) or from rumen-degradable protein (APDN) were calculated following Agroscope (2019), using the feeds' concentrations of DM, total ash, crude protein and crude fibre. Apparent total tract nutrient digestibility was estimated according to Leiber et al. (2015a). Assumptions made were a total DM intake of 20 kg/cow and day using a regression of Agroscope (2019) in relation to average milk yield of the cows. The DM intake during grazing on pasture was estimated from total DM intake minus grass, hay and pellet intakes in barn, which were determined by test weightings of the feed offered. Furthermore, ADL was assumed to be indigestible (Jung and Allen, 1995). The following equations were used:

(1) Faeces amount [kg DM/day] = ADL intake [g/day] / ADL in faeces [g/kg DM],

(2) Faecal nutrient excretion [g/day] = faeces amount [kg DM/day] × nutrient in faeces [g/kg DM],

(3) Apparent total tract nutrient digestibility [g/100 g] = (1 – (nutrient excretion [g/day]) / nutrient intake [g/day])) ×100.

From urine data, four ratios were calculated:

(1) PD:CR ratio = (allantoin [mmol/l] + uric acid [mmol/l]) / CR [mmol/l],

(2) adjusted PD:CR index = (allantoin [mmol/l] + uric acid [mmol/l]) / CR [mmol/l] × body weight [$kg^{0.75}$],

(3) PD:N ratio = PD [mmol/l] / N [g/l],

(4) N:CR ratio = nitrogen [g/l] / CR [mmol/l].

The PD:CR ratio and index are indicators for the ruminal microbial protein synthesis (Chizzotti et al., 2008). The PD:N ratio indicates the efficiency of feed N use for rumen microbial growth (Tas and Susenbeth, 2007), and the N:CR ratio is an indicator for the N excretion with the urine (Chizzotti et al., 2008).

Statistical analysis

Data were analyzed with SPSS® (Version 24) statistical software (IBM Analytics, Zurich, Switzerland), using a general linear model with treatment (level of chestnut tannins in pellets), week (1 or 3) and their interaction as fixed factors, where the data from week 0 was used for weighted least squares-correction on animal level. As no significant interaction between treatment and week occurred (except for pellet intake) and no significant week effects could be detected, tables only display treatment means and *P*-values across both sampling weeks. With the Tukey's method, multiple comparisons among treatment means were accomplished.

Results

The crude protein content of pasture and barn-fed fresh forage was highest at the start of the experiment, and decreased towards the end of the experiment (Table 1). The APDN:APDE ratio concomitantly declined from 1.16 and 1.15 (weeks 0 and 1) to 1.07 in week 3. The experimental pellets offered to the different treatment groups clearly differed in the contents of phenolic fractions, whereas they were similar in the other constituents. The amount of TEP and TT in the forage varied little throughout the experiment, and no CT could be detected. With 16.2 and 53.3 g/kg, the concentration of TT in the TAN50 and TAN100 pellets was lower than targeted.

The intake of the TAN100 pellets was significantly lower than in the other treatments (Table 2). This was more pronounced in week 1 (96.8, 82.1 and 57.9% of pellets offered consumed in TAN0, TAN50 and TAN100, respectively) than in week 3 (98.9, 99.3 and 87.1%), when there was only a tendency ($P < 0.10$) of a difference in pellet intake. Daily eating time, rumination time and number of activity changes were not influenced by dietary treatments.

Milk yield and milk solid composition also did not differ among treatments (Table 3), except for lactose content, which was lower in TAN50 compared to TAN0 and TAN100.

Faeces composition and apparent total tract digestibility estimates were not different between treatments (Table 4). Particle size distribution in faeces differed only in the proportion of the fraction >4 mm, which was significantly higher in TAN50 than TAN100. Total N concentration and PD:CR ratio in urine were greater in TAN50 than TAN0. Urinary allantoin concentration tended ($P < 0.10$) to be higher in TAN50 than TAN100. The other urine parameters did not differ between treatments.

Table 1. Chemical composition of the basal diet components (g/kg dry matter; means ± standard deviation) in the sampling weeks (mean of two samples with three replicates per sample) and ryegrass pellets including 100 g/kg of molasses, and 0, 50 and 100 g/kg chestnut tannin extract (mean of three samples with three replicates per sample)

Indices	Fresh grass-clover mixture fed in barn			Pasture forage			Grass hay fed in barn	Treatment pellets		
	0	1	3	0	1	3	3	TAN0	TAN50	TAN100
	week			week			week			
Proportion in diet, %[1]	31.7	29.0	39.0	68.3	61.6	36.2	15.1			
Analyzed variables										
dry matter, g/kg fresh weight	191 ± 8	191 ± 11	174 ± 11	221 ± 27	260 ± 5	312 ± 95	916 ± 0	895 ± 3	888 ± 4	879 ± 4
total ash	103 ± 8	107 ± 1	95.2 ± 1.5	97 ± 0	98 ± 3	93 ± 9	126 ± 23	129 ± 2	125 ± 4	118 ± 2
crude protein	155 ± 4	148 ± 2	113 ± 1	156 ± 5	141 ± 11	141 ± 7	111 ± 2	209 ± 3	207 ± 1	211 ± 1
neutral detergent fibre	377 ± 17	372 ± 45	388 ± 3	382 ± 12	391 ± 18	402 ± 35	490 ± 11	225 ± 57	235 ± 34	239 ± 31
acid detergent fibre	206 ± 8	204 ± 9	255 ± 4	193 ± 10	202 ± 14	206 ± 1	311 ± 7	155 ± 22	169 ± 17	170 ± 9
acid detergent lignin[2]	33.9 ± 0	21.7 ± 0	20.9 ± 1	19.2 ± 0	17.3 ± 0	16.5 ± 1	23.1 ± 1	76.5 ± 6	80.9 ± 2	82.2 ± 6
crude fibre	252 ± 1	258 ± 6	260 ± 1	254 ± 6	260 ± 2	261 ± 6	295 ± 6	223 ± 6	242 ± 2	254 ± 2
total extractable phenols[2,3]	26.7 ± 0	28.0 ± 3	29.3 ± 1	23.6 ± 1	23.3 ± 4	21.5 ± 2	11.9 ± 1	17.2 ± 3	33.9 ± 4	78.2 ± 4
non-tannin phenols[2,3]	19.7 ± 2	16.8 ± 2	20.7 ± 1	16.1 ± 1	19.2 ± 2	18.5 ± 1	11.2 ± 3	17.2 ± 1	17.8 ± 1	25.0 ± 1
total tannins[2,3,4]	7.1 ± 2	11.2 ± 3	8.6 ± 1	7.5 ± 2	4.1 ± 3	3.0 ± 0	0.6 ± 1	0.0 ± 0	16.1 ± 4	53.3 ± 5
condensed tannins[2,5]	ND	ND	ND	ND	ND	ND	ND	ND	0.70 ± 0	0.96 ± 0
Calculated variables										
NEL, MJ/kg dry matter	5.02 ± 0	4.85 ± 0	4.67 ± 0	5.02 ± 0	4.97 ± 0	4.86 ± 0	3.93 ± 0	5.23 ± 0.05	5.04 ± 0.05	4.89 ± 0.03
APDE	86.2 ± 0	83.5 ± 1	75.7 ± 1	85.8 ± 1	82.5 ± 2	82.4 ± 0	68.3 ± 4	94.3 ± 1	92.3 ± 0	91.3 ± 1
APDN	99.7 ± 3	94.7 ± 1	71.3 ± 1	99.8 ± 3	89.7 ± 7	90.0 ± 5	70.7 ± 1	134 ± 2	133 ± 0	135 ± 1

APDN – absorbable protein at the duodenum consisting of rumen-undegradable protein; APDE – microbial protein from fermentable energy/rumen-degradable protein; NEL – net energy for lactation; ND – not detected; [1] the difference to 100% is the proportion of fed pellets in week 1 and 3; [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; [5] leucocyanidin equivalents

Table 2. Effect of feed supplements containing different amounts of chestnut tannin extract on eating and rumination time as well as number of activity changes averaged across two experimental sampling

Indices	Treatment pellets			SEM	*P*-value
	TAN0	TAN50	TAN100		
Pellet intake, % of offered	98.5[a]	89.8[a]	74.6[b]	1.69	< 0.001
Eating time[1]					
min/day	584	583	608	9.9	0.509
5:00–13:00 h, min/h	29.7	28.1	34.1	1.40	0.203
13:00–21.00 h, min/h	32.3	33.2	39.2	2.14	0.351
21:00– 5:00 h, min/h	10.2	11.1	11.3	0.45	0.567
Rumination time[1]					
min/day	527	545	558	6.3	0.139
5:00–13:00 h, min/h	17.0	19.2	20.7	1.22	0.449
13:00–21:00 h, min/h	18.0	17.8	20.6	0.88	0.348
21:00– 5:00 h, min/h	31.3	31.2	37.1	1.46	0.159
Activity changes[1]					
n/day	130	120	122	4.1	0.527
5:00–13:00 h, *n*/h	5.31	4.82	6.14	0.432	0.454
13:00–21:00 h, *n*/h	5.74	4.87	5.99	0.350	0.423
21:00– 5:00 h, *n*/h	5.09	5.16	5.51	0.280	0.799

[a,b] – means with different superscripts differ significantly at $P < 0.05$; [1] mean of six measurements per group (seven with 100 g extract / kg) with total recording time of 72 h

Table 3. Effect of feed supplements containing different amounts of chestnut tannin extract on milk yield and composition averaged across two experimental sampling weeks

Indices	Treatment pellets			SEM	*P*-value
	TAN0	TAN50	TAN100		
Daily milk yield					
total milk, kg/cow/day	25.7	24.3	25.4	0.51	0.520
fat, g/cow/day	872	873	896	0.0	0.803
protein, g/cow/day	801	792	797	0.0	0.969
lactose, kg/cow/day	1.21	1.19	1.21	0.024	0.900
urea, g/cow/day	4.73	4.55	4.47	0.155	0.767
Milk composition					
fat, g/100 g milk	3.63	3.67	3.57	0.044	0.685
protein, g/100 g milk	3.30	3.32	3.24	0.029	0.490
lactose, g/100 g milk	4.74[a]	4.88[b]	4.77[a]	0.018	0.005
urea, mg/dl	18.3	18.1	18.2	0.563	0.985

[a,b] – means with different superscripts differ significantly at $P < 0.05$

Table 4. Effect of feed supplements containing different amounts of chestnut tannin extract on faecal and urine variables averaged across two experimental sampling weeks

Indices	Treatment pellets			SEM	*P*-value
	TAN0	TAN50	TAN100		
Faeces composition, g/kg of DM[1]					
crude protein	163	162	165	1.1	0.680
neutral detergent fibre	409	398	413	4.7	0.460
acid detergent fibre	397	379	383	4.4	0.201
acid detergent lignin	164	162	166	1.5	0.620
Estimated apparent digestibility, %					
crude protein	81.7	81.2	81.1	0.00	0.395
neutral detergent fibre	83.9	83.9	83.7	0.00	0.382
acid detergent fibre	72.4	73.2	73.4	0.00	0.306
Particles in faeces, g/100 g of DM[1]					
∑ particles > 0.3 mm	46.4	49.4	44.9	2.43	0.743
> 0.3 to 1 mm	30.8	31.8	29.8	1.36	0.842
> 1 to 2 mm	7.33	6.68	7.49	0.549	0.816
> 2 to 4 mm	4.73	5.73	4.63	0.420	0.496
> 4 mm	4.08^{ab}	5.79^{a}	3.69^{b}	0.354	0.034
Urine variables					
total N, g/l	7.12^{a}	9.31^{b}	8.72^{ab}	0.295	0.015
allantoin, mmol/l	12.7	13.8	12.2	0.29	0.063
uric acid, mmol/l	0.917	0.975	0.898	0.0220	0.327
creatinine (CR), mmol/l	4.47	4.24	4.16	0.116	0.533
purine derivatives (PD):CR ratio	3.14^{a}	3.58^{b}	3.35^{ab}	0.069	0.038
PD:CR index[2]	421	448	430	9.6	0.505
PD:N ratio, mmol/g	2.60	2.54	1.58	0.236	0.175
N:CR ratio, g/mmol	1.86	2.26	2.32	0.123	0.297

^{a,b} – means with different superscripts differ significantly at $P < 0.05$; [1] means per group, made of four samples per cow and week; [2] adjusted for body weight of individual cows

Discussion

Suitability of the on-farm approach. It was the main goal to investigate, if supplementing pellets enriched with chestnut tannin extract is a method to be established on farm in order to improve protein digestion and metabolism under conditions of dietary N excess in pasture-only feeding situations. For this purpose, an organic dairy farm with a zero-concentrate feeding strategy at spring season was chosen and the results would apply for these conditions. Limitations of an on-farm situation are that, except for yield of milk and milk constituents, only few quantitative measures are available and therefore estimates with restricted accuracy have to be used. Although data of the chewing sensors used are related with feed intake (Rombach et al., 2018), they are useful for relative estimation of treatment effects rather than absolute intake quantification under practical conditions (Leiber et al., 2016). Also regarding digestibility, only rough estimates could be calculated, which represent rather relative than absolute values. Still, there is quite a set of on-farm parameters, including the urinary N compounds, which may give information about the influence of the chestnut tannin extract on the nutrient digestion and protein metabolism of the dairy cows. The important advantage of this kind of on-farm experiments is that results are closer to real practice conditions than it might be obtained on station.

Experimental feeds. In the present study, pellets with increasing contents of chestnut tannin extract, providing approximately 0, 5 and 10 g extract per kg of total diet, were fed during 21 days. Unfortunately, this resulted in clearly lower measured tannin concentrations than had been targeted (0, 1.6 and 5.3 g total tannins, respectively, as analyzed). Considering also the incomplete intake of the pellets (90% for TAN50, 75% for TAN100), the realized intake was only 29 g of hydrolysable tannin/day for TAN50 and 80 g/day for TAN100. However, with these concentrations of chestnut tannins in the diet, effects on N metabolism in ruminants had been achieved in other studies (Ali et al., 2017; Aboagye et al., 2018). For on-farm conditions a risk of detrimental effects due to too high tannin supplements (Jayanegara et al., 2011; Henke et al., 2017) cannot be accepted. Therefore, even though the realized tannin levels were at the lower end, it must be considered that, given an obvious difficulty of exact dosage, there would be not much margin to increase them without negative effects. Thus, if the actual supplementation levels in this study would not work, this would mean a fail of concept for practice.

Although milk urea concentrations were not high, there was still potential to reduce them. The basal diet indeed had an excess of crude protein with APDN > APDE and CP/NEL > 25, suggesting that crude protein supply for the rumen microbial synthesis was too high in relation to their energy supply.

Palatability, intake and milk production. The immediate palatability of the pellets was limited, especially with the highest level of chestnut extract. In week 1, only slightly more than half of the amount offered in TAN100 was consumed, but even with treatment TAN50, 20% of offered amount was refused. This was likely owed to the astringent taste of tannins (Kumar and Singh, 1984) as, in order to limit need for pellets, extract concentration was quite high. In other studies, the extract was given *via* concentrate (e.g., Ali et al., 2017) or mixed in total

ration or silage (Śliwiński et al., 2004; Colombini et al., 2009; Aboagye et al., 2018) to ensure high and stable intake. These approaches, however, were impossible under the given on-farm conditions.

Regarding total feed intake indicators, we found no significant differences in eating and rumination time among groups and the data recorded was in the expected range (Rombach et al., 2018). Therefore, it could be assumed that total intake on pasture and during the night in the barn did not substantially differ among TAN0, TAN50 and TAN100, and that there was no effect on rumination time. Supplementing chestnut tannin extract did also not affect milk yield. This is consistent with some other studies, where even higher concentrations of chestnut tannins were used (Śliwiński et al., 2004; Colombini et al., 2009). Contrary to the literature (Śliwiński et al., 2004; Colombini et al., 2009; Ali et al., 2017), in the present study the protein and fat concentrations were not affected. Most importantly, falsifying part of the hypothesis, no effects on milk urea occurred. The results indicate that the supplementation level chosen was likely too low to provoke the desired effects. However, higher tannin supplementation levels could also have led to milk yield depressions (Henke et al., 2017), which has to be avoided in practice. Thus, it appears to be very critical to find the right dosage of tannins if clear but only positive effects on protein efficiency are the target.

Indicators of chestnut extract effects in faeces and urine. Purine derivatives in the urine allow for estimation the amount of rumen microbial protein produced and digested in the duodenum, as they origin mainly from rumen microbial nucleic acids and their derivatives (Tas and Susenbeth, 2007; Henke et al., 2017). In the present study, almost no effects of treatment on these indicators were found, which is in line with the absence of effects on milk urea, and indicates once again that even 5.3 g hydrolysable tannins per kg DM of feed (as realized with TAN100) is below the effective level. The urinary N concentrations and excretion in ruminants partly depend on the amount of ammonia formed in the rumen, which increases with surplus of protein available to the rumen microbes in diet compared to their energy supply (Nousiainen et al., 2004). When urinary N concentration was related to CR concentrations to correct for dilution in spot samples (Chizzotti et al., 2008), this parameter was also not significantly different between groups. Also, the N content of faeces was not influenced by supplementing the chestnut extract. This was also opposite to our expectation, that tannins would shift the N excretion from urine to faeces (Mueller-Harvey, 2006). The apparent total tract nutrient digestibility estimates had been calculated under the assumption, that DM intake was adequately estimated and that ADL is fully indigestible (Jung and Allen, 1995). These digestibility estimates were not affected by treatment. Also, no effects were found on faecal fibre fractions, which are considered to be indicative for fibre degradation (Leiber et al., 2015b). This indicates that no detrimental effects on digestion were provoked by the given chestnut tannin supplementation levels.

Conclusions

From a theoretical concept, supplementing ryegrass pellets enriched with chestnut tannin extract in a low-input system at the times of excessive dietary crude protein could be quite easily realized on commercial farms. The present study, however, revealed two major setbacks. Even at a dosage too low to reach the desired effects, palatability of the pellets was impaired. Despite this, no clear improvements in N utilization of the cows were found. Based on these results, the supplementation of hydrolysable tannins to dairy cows in low-input systems does not appear to improve protein digestion and N metabolism, and is therefore not recommended.

Acknowledgments

We thank to Alois Huber, Wildegg, for giving access to his cows and the laboratory staff of FiBL and University of Hohenheim for supporting analyses. This project was funded by the Swiss National Science Foundation (project no. 31003A_166425).

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