# Investigation of sainfoin (Onobrychis viciifolia) cultivar differences on nitrogen balance and fecal egg count in artificially infected lambs

Azuhnwi, B N ; Hertzberg, H ; Arrigo, Y ; Gutzwiller, A ; Hess, H D ; Mueller-Harvey, I ; Torgerson, P R ; Kreuzer, M ; Dohme-Meier, F

DOI: https://doi.org/10.2527/jas.2012-5351

# Investigation of sainfoin (Onobrychis viciifolia) cultivar differences on nitrogen balance and fecal egg count in artificially infected lambs

B. N. Azuhnwi, H. Hertzberg, Y. Arrigo, A. Gutzwiller, H. D. Hess, I. Mueller-Harvey, P. R. Torgerson, M. Kreuzer and F. Dohme-Meier

*J ANIM SCI* 2013, 91:2343-2354.
doi: 10.2527/jas.2012-5351 originally published online March 5, 2013

# Investigation of sainfoin (*Onobrychis viciifolia*) cultivar differences on nitrogen balance and fecal egg count in artificially infected lambs[1]

**B. N. Azuhnwi,*† H. Hertzberg,‡ Y. Arrigo,* A. Gutzwiller,* H. D. Hess,* I. Mueller-Harvey, § P. R. Torgerson,# M. Kreuzer,† and F. Dohme-Meier*[2]**

*Agroscope Liebefeld-Posieux Research Station ALP, Tioleyre 4, 1725 Posieux, Switzerland; †ETH Zurich, Institute of Agricultural Sciences, Zurich, Switzerland; ‡Institute of Parasitology, Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland; §Chemistry and Biochemistry Laboratory, School of Agriculture, Policy, and Development, University of Reading, 1 Earley Gate, P.O. Box 236, Reading RG6 6AT, United Kingdom; and #Section of Epidemiology, Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland

**ABSTRACT:** Research in ruminant nutrition and helminth control with forages, which contain condensed tannins (CT), suggests that varying responses may depend not only on CT concentration but also on CT composition. An experiment was designed to test this by feeding 2 dried sainfoin cultivars (Visnovsky and Perly), which differed in CT properties, to lambs that were artificially infected with the abomasal blood-sucking nematode *Haemonchus contortus*. Twenty-four infected lambs received 1 of these 2 cultivars; the feeds were either untreated or treated with the CT-binding polyethylene glycol over 4 wk ($n$ = 6). The 2 cultivars were also fed to 2 × 6 uninfected lambs. Nutrient digestibility, N balance, ADG, plasma urea, together with indicators of infection [fecal egg count (FEC), abomasal worm count, per capita female fecundity, erythrocytic indices, and serum protein], were determined. The specific effects of sainfoin cultivar, CT, and infection were evaluated by contrast analysis. Digestibility of both NDF and ADF were less ($P$ < 0.001) with Perly compared with Visnovsky. The apparent nutrient digestibility was reduced ($P$ < 0.001) by CT. However, no clear cultivar effects were evident on N excretion and retention. Condensed tannins reduced ($P$ = 0.05) body N retention and shifted ($P$ < 0.001) N excretion from urine to feces. Unlike cultivar and CT, infection decreased ($P$ = 0.002) ADG. Plasma urea concentration was decreased ($P$ = 0.007) in Perly- compared with Visnovsky-fed lambs and was decreased ($P$ < 0.001) by CT. Plasma concentrations of essential and semiessential AA were increased ($P$ < 0.001) by CT. The groups of infected lambs did not clearly differ in abomasal worm counts and erythrocytic indicators. In the last 2 to 3 wk of the experiment, FEC was decreased ($P \leq 0.01$) when feeding CT. The lack of substantial cultivar effects suggests that the differences in CT properties may have been too small to result in nutritional and anthelmintic effects. The present results indicate that sainfoin CT had a mitigating effect on FEC and, consequently, pasture infectivity. However, the reduction was too small to expect any significant benefits in an *Haemonchus*-dominated system. Therefore, the use of sainfoin for controlling *H. contortus* should only be one component within an integrated worm control system.

**Key words:** condensed tannin composition, *Haemonchus contortus*, N balance, polyethylene glycol, sainfoin, sheep



J. Anim. Sci. 2013.91:2343–2354
doi:10.2527/jas2012-5351

## INTRODUCTION

The excessive and wasteful breakdown of feed proteins in temperate ruminant production systems, and also the increased resistance of parasitic nematodes to currently available anthelmintics give rise to ecological and economical concerns; therefore, the search is on

[1]The authors acknowledge the financial support from the Swiss Grassland Society (AGFF), Zurich, Switzerland. They are grateful for the excellent laboratory support and care of the animals by the staff of ALP, Posieux, Switzerland; and thank Delley Seeds and Plants Ltd. (DSP), Delley, Switzerland, and Eric Schweizer AG, Thun, Switzerland, for providing the seeds of the cultivars Perly and Visnovsky, respectively.

[2]Corresponding author: frigga.dohme-meier@agroscope.admin.ch

Received April 3, 2012.
Accepted February 12, 2013.

for alternative approaches (Mueller-Harvey, 2006). Forages with moderate levels of condensed tannins (**CT**) such as sainfoin (*Onobrychis viciifolia* Scop.) are believed to offer an attractive option in this respect (Min et al., 2003). However, controversy still exists as to the actual beneficial effects of sainfoin. Waghorn (2008), reviewing the effects of dietary CT in forage legumes for sustainable ruminant production, ascribed no beneficial effect to sainfoin in productivity other than mitigating the impact of parasites. Also, the outcomes of several experiments were inconsistent in the effects of sainfoin on ruminant nutrition and gastrointestinal parasite control (Heckendorn et al., 2006; Scharenberg et al., 2007; Aufrère et al., 2008; Scharenberg et al., 2008). Scientific evidence suggests that not only quantitative (i.e., differences in CT concentration) but also qualitative (i.e., differences in CT composition) traits should be considered when exploring the varying responses by ruminants to sainfoin feeding (Min et al., 2003; Theodoridou et al., 2010, 2011).

In the present experiment, we tested 2 hypotheses: i) the nutritional and anthelmintic effects of sainfoin vary with CT content and CT composition, and ii) the nutritional and anthelmintic effects result from the CT in sainfoin. Experimentally, the CT activity was neutralized with polyethylene glycol (**PEG**), which forms a complex with CT. Visnovsky and Perly were chosen as the sainfoin cultivars, as these had been shown previously to differ in CT concentration (Azuhnwi et al., 2011) and composition (Azuhnwi et al., 2011). Growing lambs were artificially infected with the abomasal blood sucking nematode, *Haemonchus contortus*.

## MATERIALS AND METHODS

All manipulations applied to the animals in the experiment were approved (No 20/10) by the Animal Care Committee of the Canton of Fribourg, Switzerland.

### *Experimental Forages*

Two adjacent field plots, each measuring 140 × 15 $m^2$ for the 2 multiple-flowering type sainfoin cultivars, Visnovsky [Eric Schweizer AG, Thun, Switzerland) and Perly (Delley Seeds and Plants Ltd. (**DSP**), Delley, Switzerland], were sown in spring of 2010 at ALP Posieux, Switzerland (46°46′ N, 07°06′ E; altitude: 650 m). The soil was sandy loam with pH of 6.2, $P_2O_5$ at 64.1 mg/kg, $K_2O$ at 150.5 mg/kg, and MgO at 93.7 mg/kg. Due to weed infestation, a topping treatment was done by cutting forage on plots in mid-June. Above ground sainfoin material was then harvested after 42 d of regrowth at the preflowering stage at about 5 cm above the soil with a FELLA drum mower (FELLA KM 310 FZ, FELLA-Werke GmbH, Feucht, Germany). At harvest, the botanical composition of the swards on the plots with Visnovsky and Perly was determined by collecting 12 representative samples from a zigzag transect on each of the plots and calculating the average composition on a fresh matter basis of sainfoin, herbs, and grasses. Drying of freshly cut sainfoin was performed by using a special drying system (Physitech, Wabern, Switzerland) as described in Scharenberg et al. (2007). Additionally, a grass-clover mixture was harvested from the first cut of a pasture. This sward was proportionally composed (fresh matter basis) of 74.2% grasses, 6.8% legumes, and 19.0% herbs. The grass-clover mixture was wilted in the field for 1 d and then dried by forced warm air in the barn.

### *Animals and Experimental Design*

The experiment started off with 42 castrated male White Alpine Sheep lambs aged between 3 and 4 mo. The animals were allocated to 3 groups of 14 lambs, each tested subsequently in 3 experimental series lasting for 8 wk each. Before that, all lambs had been drenched with an anthelmintic drug (7.5 mg of levamisole/kg of BW; 10 mg triclabendazole/kg of BW; Endex, Novartis AG, Basel, Switzerland) and had received an intramuscular injection (0.2 mg of sodium selenite/kg of BW and 5 mg of vitamin E/kg of BW; Dr. E. Gräub AG, Berne, Switzerland). Additionally, an anticoccidial treatment (20 mg toltrazuril/kg of BW; Baycox 5%, Provet AG, Burgdorf, Switzerland) was applied.

At the start of each experimental series, infective larvae ($L_3$) of *H. contortus* in tap water were administered orally to 9 of the 14 lambs with a syringe at a dose of 250 larvae/kg BW. During the first 4 wk of the experiment, infected and uninfected lambs were housed separately in groups in straw-bedded boxes and had ad libitum access to a dried grass–clover mixture (DM basis: 89% OM, 18.9% CP, 45.5% NDF, 24.5% ADF, and 17.4 MJ GE) and to fresh water. Additionally, twice daily 10 g each of a commercial mineralized salt mix (UFA 998, UFA, Herzogenbuchsee, Switzerland) was offered to the sheep in common salt-feeding troughs. To avoid reinfection, straw of the infected sheep was changed weekly.

The infection had been established during wk 4. Then 8 of the 9 infected lambs per series were selected and allocated by BW and fecal egg counts (**FEC**) to 4 different treatments, which were then applied for the next 4 wk. The treatments consisted of the sainfoin cultivar Visnovsky either untreated (**Vi**) or treated with PEG (**Vi-PEG**) and the sainfoin cultivar Perly either untreated (**Pi**) or treated with PEG (**Pi-PEG**). From the remaining 5 uninfected lambs per series, 4 were selected and

grouped by BW to the last 2 treatments where Visnovsky (**Vu**) and Perly (**Pu**) were fed. Overall, this resulted in 6 lambs per treatment, with allocation done in such a way as to have similar BW across all 6 treatments: Vi, 25.3 ± 3.9 kg; Vi-PEG, 24.4 ± 2.6 kg; Vu, 25.9 ± 3.4 kg; Pi, 25.5 ± 2.6 kg; Pi-PEG, 25.6 ± 0.9 kg; Pu, 24.8 ± 2.2 kg (means ± SD). The lambs excluded from the experiment were slaughtered when they reached their slaughter BW. Polyethylene glycol was applied by pouring 200 mL of an aqueous 25% (wt/vol) solution (polyethylene glycol 4000, Roth, Karlsruhe, Germany) over each meal 9 h before feeding. This corresponded to 100 g/d of PEG 4000 (Scharenberg et al., 2008), calculated to obtain a minimum ratio of PEG:CT of 1:1 and ensured that all CT from sainfoin were neutralized. From wk 5 to 8, the experimental diets were allocated in amounts calculated to supply 66 g of OM/kg of metabolic BW ($BW^{0.75}$) daily from the forage as the amount estimated to meet the energy requirements of the lambs (Agroscope Liebefeld-Posieux, 2010) and, at the same time, to ensure a similar and mostly complete intake relative to BW in all lambs in all series. Feeding levels were adjusted weekly based on the actual BW of the lambs. Feeding times were 0730 and 1630 h. A same quantity of 20 g/d of the mineralized salt mix as in the first 4 wk was offered for 30 min, always before forage feeding. During the entire experiment, sheep had free access to fresh water. Because of the rapidly falling packed cell volume (**PCV**) in infected sheep, lambs in all 6 treatment groups received an intramuscular injection of iron dextran (300 mg of $Fe^{3+}$, Ferriphor, Dr E. Gräub AG, Bern, Switzerland) once in wk 5.

Treatment feeding during wk 5 to wk 7 was used as an adaptation period to the experimental diets. In that time, the lambs were housed in individual boxes on sawdust. During wk 8 of experiment (collection period), the sheep were kept in metabolic crates constructed to allow the complete and separate collection of urine and feces. Before the beginning of the collection period, the animals had been accustomed to this procedure by putting them into the crates for 2 d. At the end of the collection period, lambs were slaughtered and the abomasa were ligated and removed to determine the luminal *H. contortus* burden.

### *Sampling Procedure*

Beginning on the day of the infection, samples of blood and feces were taken once weekly at 2 h after the morning feeding to monitor the course of the infection by measuring PCV, hemoglobin (**HB**) concentration, mean corpuscular volume (**MCV**), mean corpuscular hemoglobin concentration (**MCHC**) in blood, and FEC. Additionally, blood samples were taken before (wk 7) and after (wk 8) the collection period for serum and plasma. Nine milliliters blood per sample were taken from a *Vena jugularis* and collected either in vacuum tubes containing Z Serum Clot activator (Greiner Bio-One, Kremsmünster, Austria) stored at ambient temperature for serum; in vacuum tubes containing $K_3$EDTA (Greiner Bio-One) for hematology; or in heparinized vacuum tubes (Greiner Bio-One) for plasma. Vacuum tubes for serum and plasma were later centrifuged at 1,500 × *g* for 15 min at 4°C and serum and plasma were harvested and stored at –20°C until analyses. During the collection periods, daily feed samples were collected and later pooled into weekly samples for determination of the chemical composition. During the collection period, feces, urine, and forage refusals were quantitatively collected once daily and water consumption was determined. Urine was collected in a vessel containing 2.5 *M* sulfuric acid and thus did not exceed a pH of 2 to 4, which prevented gaseous N losses. Feces and urine samples were stored at –20°C. At the end of the collection period, the daily samples of feces, urine, and forage refusals were pooled proportionately to the total amounts across the collection period for every lamb. In the PEG-treated lambs, digestibility was calculated with corrections of OM and GE due to PEG additions and assuming a uniform distribution of PEG within forage.

The ligated abomasa of the slaughtered lambs were stored on ice for a maximum of 24 h, subsequently opened and thoroughly washed over a 200-µm sieve. Luminal contents were collected and preserved in 5% (vol/vol) formalin.

### *Laboratory Analyses*

Feed refusals and feces were lyophilized, and ground to pass a 1-mm screen (Brabender mill, no. 880804, Brabender, Duisburg, Germany). The DM contents of these samples and of PEG were measured (3 h at 105°C). Total ash was then determined by dry-ashing at 550°C for 4 h. Cell wall constituents were analyzed using an Ankom 200/220 Fiber Analyzer (Ankom Technology Corporation, Fairport, NY). Neutral detergent fiber was determined with the addition of heat-stable amylase and sodium sulfite and corrected for residual ash after incineration at 500°C for 1 h (aNDFom, Mertens, 2002). Acid detergent fiber (AOAC, 1995; 973.18) was also determined and corrected for residual ash (ADFom). The N contents of feeds, refusals, feces, and urine were measured by the Dumas method using a C/N analyzer (FP-2000, Leco Instruments, St. Joseph, MI; AOAC, 1995; method number 968.06). The CP content was calculated as 6.25 × N content. For determination of AA, feed samples were prepared as recommended by the European Union (1998), including digestion with

HCl, and analyzed by HPLC (Alliance 2695, Waters, Milford, MA) following the manufacturer's manual (Waters AccQ Tag Chemistry Package 052874 TP, rev. 1). A LECO Isoperibol Bomb Calorimeter System (AC-350, LECO Instruments, St Joseph, MI) was used to analyze GE. Total CT were determined in lyophilized feed samples using the method of Terrill et al. (1992). For each feed sample, duplicate portions of 500 mg were used and the absorbance of the resulting anthocyanidins was read at 550 nm on a UV/VIS Spectrometer (PerkinElmer, Schwerzenbach, Switzerland) with the use of appropriate blanks to account for background absorbance. The CT standard used for determination of CT concentration was prepared from the Visnovsky cultivar based on the method of Stewart et al. (2000). The mean degree of polymerization (**mDP**), the procyanidin (**PC**)/prodelphinidin (**PD**) ratio, and the *cis*/*trans* flavanol ratio were obtained by direct thiolysis of CT in feed samples in the presence of benzyl mercaptan followed by analysis of thiolytic degradation products on a Gilson HPLC system (Anachem Instruments, Luton, UK) using the protocol of Gea et al. (2011).

Fecal egg counts were measured according to Heckendorn et al. (2006). The DM content of feces was determined in a 3-g subsample dried in a forced-air oven at 105°C for 16 h. The FEC were expressed as numbers of eggs per gram of dried feces (Heckendorn et al., 2006). Adult worm counts and sex identification were performed microscopically in a 10% aliquot of the overall abomasal content at 12-fold magnification. Per capita female fecundity was calculated by dividing the individual FEC at slaughter by the total number of female worms recovered for each lamb (Heckendorn et al., 2006).

Hemoglobin, PCV, MCV, and MCHC were determined on whole blood with an automated system (Poch100iV, Sysmex, Horgen, Switzerland). Albumin, total protein (ALB plus, Total Protein, Roche Diagnostics, Basel, Switzerland), glucose (**GLU**; Gloco-quant, Roche Diagnostic), and urea (UV 250, bioMérieux, Geneva, Switzerland) were analyzed in blood serum with test kits. Urinary urea was analyzed, after enzymatic treatment with urease and glutamate dehydrogenase (UV 250, bioMérieux) on an autoanalyzer (COBRAS Mira, Roche Diagnostics; standard: Calimat, bioMérieux). For the determination of the free plasma AA, nor-leucine (No. 74560, Fluka-Chemie AG, Buchs, Switzerland) was added as internal standard. Proteins were precipitated with 75% acetonitrile (No. 00692, Fluka-Chemie AG). The supernatant was dried in a Speedvac and dissolved in coupling buffer (75% methanol, No. 65541, Fluka-Chemie AG), 10% triethylamine (No. 25108, Thermo Scientific, Lausanne, Switzerland), and 1% phenylisothiocyanate (**PITC**, No. P-1034, Sigma-Aldrich Chemie, Buchs, Switzerland) in water. The mixture was agitated for 20 min for complete derivatization of all AA before drying in a Speedvac. The pellet was dissolved in Buffer A (5.5 g sodium dihydrogen phosphate monohydrate in water, pH 7.2). The derivatized AA were separated on a Zorbac Eclipse AA (3.5 µm, 150 × 3 mm, No. 961400-302, Agilent, Basel, Switzerland) column with a gradient elution from 97% Buffer A to 45% Buffer B (45% methanol, 45% acetonitrile in water) in 60 min, detected at 247 nm, and quantified using an external standard solution (No. 5061-3330, Agilent Technologies, Schweiz AG, Basel, Switzerland) supplemented with phosposerine (No. 79710, Fluka-Chemie AG), citrulline (No. 27510, Fluka-Chemie AG), tryptophan (No. 8374, Merck (Schweiz) AG, Zug, Switzerland), glutamine (No. 289, Merck (Schweiz) AG), and asparagine (No. 11159, Fluka-Chemie AG).

### *Statistical Analyses*

Data were subjected to ANOVA using the GLM procedure (SAS Inst. Inc., Cary, NC), with treatment, series, and their interactions as fixed factors. Contrasts were used to specifically determine i) the cultivar effect by comparing all Visnovsky treatments against the Perly treatments (Vi, Vi-PEG, Vu vs. Pi, Pi-PEG, Pu); ii) the CT effect in infected animals by comparing treatments with and without PEG (Vi, Pi vs. Vi-PEG, Pi-PEG); iii) the effect of *H. contortus* infection by comparing treatments with infected and with uninfected lambs (Vi, Pi vs. Vu, Pu). Statistical significance was considered when $P \leq 0.05$, whereas a $P > 0.05$ to $\leq 0.1$ was considered as a trend. Values of PCV, HB, MCV, and MCHC in blood were analyzed with the MIXED procedure of SAS using the repeated measurement statement fitting an autoregressive covariance structure, because each of these variables were based on observations obtained at different time points. The corresponding model included treatment, week, and their interaction as fixed factors and series as a random factor. The effects on FEC in the infected lambs, where measurements began with the first egg excretion found 2 wk postinfection, per capita female fecundity, and adult worm counts were analyzed using R software v. 2.13.2 (R Development Core Team, 2011). There was evidence of aggregated distributions, that is, variance being greater than the mean (Torgerson et al., 2005), for FEC, per capita female fecundity, and adult worm counts. Cross-sectional negative binomial regression models were therefore fitted separately for each point in time, with the 2 parameters of the model being the arithmetic mean and the negative binomial constant for each of the 3 indices. The mean FEC, per capita female fecundity, adult worm counts, and the 95% negative binomial confidence intervals were estimated by maximum likelihood techniques. In these variables,

comparisons were made only between the various infected treatments by comparing Vi vs. Pi; Vi vs. Vi-PEG; Pi vs. Pi-PEG; Vi-PEG vs. Pi-PEG. Generally, $P \leq 0.05$ was considered significant. To test the relationship between CT properties and parasitological variables, Pearson's correlation coefficients were established between intake of PD and intake of CT with adult worm counts and FEC at the end of the experiment using data from infected lambs of treatments Vi and Pi.

## RESULTS

### *Chemical Composition of Feeds*

The Visnovsky sward consisted of 64.3% sainfoin, 30.8% herbs, and 4.9% grasses (fresh matter basis; data not shown). The Perly plot contained 71.2% sainfoin, 28.0% herbs, and 0.8% grasses. The CT concentrations of the Visnovsky and Perly swards were 8.1% and 9.7% of DM, respectively. The structural composition of CT of the 2 cultivars was described by the mDP, the PC/PD ratio, and the *cis*/*trans* ratio and is given in Table 1 together with the contents of OM, CP, NDF, ADF, GE, and AA.

**Table 1.** Composition of 2 dried sainfoin cultivars during the collection periods ($n = 3$, mean ± SD)

| Item | Visnovsky | Perly |
|---|---|---|
| Analyzed composition, % of DM | | |
| OM | 88.0 ± 0.79 | 90.1 ± 0.51 |
| CP | 19.4 ± 0.81 | 19.2 ± 0.59 |
| NDF | 37.2 ± 3.28 | 34.1 ± 1.80 |
| ADF | 31.5 ± 2.21 | 28.8 ± 0.81 |
| GE (MJ/kg DM) | 17.3 ± 0.15 | 17.9 ± 0.23 |
| Condensed tannins (CT)[1] | 8.1 ± 0.33 | 9.7 ± 0.21 |
| Structural composition of CT[2] | | |
| Mean degree of polymerization | 29.1 ± 1.64 | 20.1 ± 3.69 |
| Procyanidin/prodelphinidin ratio | 24/76 ± 5.6 | 37/63 ± 3.2 |
| Cis/trans ratio | 88/12 ± 3.3 | 84/16 ± 0.6 |
| Total AA analyzed (% of DM) | 16.3 ± 0.14 | 16.4 ± 0.15 |

[1]Analyzed by the butanol-HCl method (Terrill et al., 1992).

[2]Analyzed by direct thiolysis-HPLC (Gea et al., 2011).

### *Intake, Digestibility, and Body Weight*

Due to refusals in the Vi group and the numerical differences in the OM and GE contents of the 2 cultivars, Visnovsky-fed lambs had a decreased (cultivar contrast: $P < 0.001$) intake of DM, OM, and GE than Perly-fed lambs (Table 2). No differences were observed between cultivars for NDF (cultivar contrast: $P = 0.12$) intakes, but ADF intakes tended to be slightly greater with Visnovsky (cultivar contrast: $P = 0.07$). Condensed tannins and infection had no effect ($P \geq 0.60$) on nutrient intake. Water consumption remained unaffected ($P \geq 0.10$) by any of the treatments. Apparent digestibility coefficients of NDF and ADF were greater for the Visnovsky than the Perly treatments (cultivar contrast: $P < 0.001$). The presence of CT reduced the apparent digestibility of all traits (CT contrast: $P < 0.001$). Infection with *H. contortus* did not influence digestibility (infection contrast: $P \geq 0.40$). Treatments had no effect on BW ($P \geq 0.23$).

**Table 2.** Effect of cultivar, condensed tannins (CT), and infection with *Haemonchus contortus* on intake, apparent digestibility, and BW ($n = 6$)

| | Treatments[1] | | | | | | | *P*-values of contrasts[2] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item | Vi | Vi-PEG | Vu | Pi | Pi-PEG | Pu | SEM | Cultivar | CT | Infection |
| Intake, g/kg of $BW^{0.75}$ daily | | | | | | | | | | |
| DM | 68.5 | 69.3 | 69.7 | 72.2 | 72.2 | 71.5 | 0.82 | <0.001 | 0.60 | 0.76 |
| OM | 60.4 | 60.6 | 61.4 | 65.0 | 64.9 | 64.4 | 0.83 | <0.001 | 0.94 | 0.83 |
| NDF | 25.2 | 24.9 | 25.4 | 24.5 | 24.3 | 24.2 | 0.65 | 0.12 | 0.75 | 0.91 |
| ADF | 21.4 | 21.1 | 21.6 | 20.7 | 20.5 | 20.4 | 0.53 | 0.07 | 0.72 | 0.92 |
| GE | 1.19 | 1.19 | 1.21 | 1.30 | 1.29 | 1.28 | 0.016 | <0.001 | 0.93 | 0.78 |
| Tap water consumption, kg/d | 3.56 | 3.38 | 3.63 | 4.17 | 4.09 | 3.09 | 0.294 | 0.28 | 0.66 | 0.10 |
| Apparent digestibility, % | | | | | | | | | | |
| DM | 61.4 | 63.2 | 60.8 | 61.0 | 64.3 | 62.3 | 0.59 | 0.13 | <0.001 | 0.55 |
| OM | 64.9 | 69.5 | 64.6 | 64.9 | 69.4 | 65.7 | 0.54 | 0.44 | <0.001 | 0.67 |
| NDF | 51.3 | 62.7 | 51.3 | 46.9 | 57.5 | 47.6 | 1.09 | <0.001 | <0.001 | 0.74 |
| ADF | 43.0 | 57.8 | 44.6 | 38.7 | 52.1 | 38.5 | 0.85 | <0.001 | <0.001 | 0.40 |
| GE | 60.5 | 66.3 | 60.4 | 61.3 | 66.3 | 61.6 | 0.61 | 0.17 | <0.001 | 0.95 |
| BW,[3] kg | 32.4 | 32.4 | 35.7 | 33.1 | 33.6 | 33.3 | 0.44 | 0.88 | 0.85 | 0.23 |

[1]Vi = infected lambs fed Visnovsky; Vi-PEG = infected lambs fed Visnovsky treated with polyethylene glycol; Vu = uninfected lambs fed Visnovsky; Pi = infected lambs fed Perly; Pi-PEG = infected lambs fed Perly treated with polyethylene glycol; Pu = uninfected lambs fed Perly.

[2]Cultivar = Vi, Vi-PEG, Vu vs. Pi, Pi-PEG, Pu; CT = Vi, Pi vs. Vi-PEG, Pi-PEG; Infection = Vi, Pi vs. Vu, Pu.

[3]Measured before and after the collection period.

**Table 3.** Effect of cultivar, condensed tannins (CT), and infection with *Haemonchus contortus* on N balance and ADG ($n = 6$)

| | Treatments[1] | | | | | | | *P*-value of contrasts[2] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item | Vi | Vi-PEG | Vu | Pi | Pi-PEG | Pu | SEM | Cultivar | CT | Infection |
| N, g/kg of $BW^{0.75}$ daily | | | | | | | | | | |
| Intake | 2.14 | 2.20 | 2.20 | 2.22 | 2.23 | 2.21 | 0.015 | 0.002 | 0.02 | 0.19 |
| Feces | 0.82 | 0.60 | 0.86 | 0.89 | 0.57 | 0.87 | 0.017 | 0.22 | <0.001 | 0.58 |
| Urine | 1.12 | 1.31 | 1.08 | 1.09 | 1.38 | 1.06 | 0.019 | 0.69 | <0.001 | 0.08 |
| Total excretion | 1.94 | 1.90 | 1.94 | 1.98 | 1.95 | 1.93 | 0.029 | 0.33 | 0.27 | 0.39 |
| Body retention | 0.20 | 0.30 | 0.25 | 0.24 | 0.28 | 0.28 | 0.025 | 0.37 | 0.01 | 0.08 |
| N, % of N intake | | | | | | | | | | |
| Feces | 38.5 | 27.1 | 39.4 | 40.2 | 25.6 | 39.4 | 0.74 | 0.89 | <0.001 | 0.90 |
| Urine | 52.2 | 59.4 | 49.3 | 48.9 | 61.8 | 47.8 | 0.74 | 0.21 | <0.001 | 0.01 |
| Total excretion | 90.7 | 86.4 | 88.7 | 89.1 | 87.4 | 87.2 | 2.86 | 0.47 | 0.02 | 0.10 |
| Urinary N, % of total N excreted | 57.6 | 68.7 | 55.6 | 54.9 | 70.7 | 54.8 | 1.07 | 0.28 | <0.001 | 0.05 |
| Urea N excretion, g/d | 31.9 | 37.6 | 32.4 | 30.1 | 39.0 | 29.7 | 1.21 | 0.31 | <0.001 | 0.94 |
| ADG,[3] g | 39.7 | 62.4 | 97.5 | 52.6 | 75.1 | 91.7 | 14.31 | 0.58 | 0.13 | 0.002 |

[1]Vi = infected lambs fed Visnovsky; Vi-PEG = infected lambs fed Visnovsky treated with polyethylene glycol; Vu = uninfected lambs fed Visnovsky; Pi = infected lambs fed Perly; Pi-PEG = infected lambs fed Perly treated with polyethylene glycol; Pu = uninfected lambs fed Perly.

[2]Cultivar = Vi, Vi-PEG, Vu vs. Pi, Pi-PEG, Pu; CT = Vi, Pi vs. Vi-PEG, Pi-PEG; Infection = Vi, Pi vs. Vu, Pu.

[3]During the entire 4 wk of sainfoin feeding.

### *Nitrogen Balance and Average Daily Gain*

Due to some refusals by the infected lambs on the Visnovsky (Vi) diet, N intake was correspondingly decreased (cultivar contrast: $P = 0.002$; CT contrast: $P = 0.02$; Table 3). Apart from this, cultivar did not influence (cultivar contrast: $P \geq 0.21$) any of the N-balance variables. Lambs fed CT excreted more fecal N (CT contrasts: $P < 0.001$) and less urinary N (CT contrasts: $P < 0.001$). Total N-excretion was unaffected (CT contrasts: $P = 0.27$), but relative to N-intake CT increased the percentage of excreted N (CT contrast: $P = 0.02$). The difference between untreated and PEG treated lambs was +32% for fecal N and –22% for urinary N. Consequently, CT increased the proportion of fecal N as percentage of N-intake and decreased urinary N (CT contrasts: $P < 0.001$). In addition, urinary N as percentage of total N excreted and the amount of urea-N excretion in urine was less (CT contrast: $P < 0.001$) in lambs fed untreated compared with those fed PEG-treated sainfoin. Condensed tannins decreased body N retention (CT contrasts: $P = 0.01$). There was a trend (infection contrast: $P = 0.08$) toward greater excretion of urinary N and decreased body N retention in infected compared with uninfected lambs. Furthermore, infection increased urinary N as a percentage of N intake (infection contrast: $P = 0.01$) and of total N excreted (infection contrast: $P = 0.05$). The ADG over the entire 4 wk period of sainfoin feeding was lower for infected compared with uninfected lambs (infection contrast: $P = 0.002$), whereas cultivar and CT had no effect ($P \geq 0.13$) on ADG.

### *Blood Variables*

Plasma urea concentration was greater in Visnovsky- compared with Perly-fed lambs (cultivar contrast: $P = 0.007$; Table 4). All other blood variables were unaffected by cultivar (cultivar contrast: $P \geq 0.13$). Feeding CT decreased ($P < 0.001$) plasma urea concentrations but did not influence (CT contrast: $P \geq 0.19$) plasma glucose, serum albumin, and protein concentrations. Furthermore, CT increased plasma Arg (trend, CT contrasts: $P = 0.06$), His, Leu, Lys, and Val (CT contrast: $P \leq 0.01$) concentrations, which resulted in an increased plasma concentration of total essential plus semiessential AA (EAA; CT contrast: $P < 0.001$) and a trend to a greater EAA:non-EAA (**NEAA**) ratio (CT contrast: $P = 0.07$). Apart from plasma Ala concentration, which was greater (CT contrast: $P = 0.05$) in the presence of CT, no other NEAA were influenced (CT contrast: $P \geq 0.24$) by CT. Infection decreased serum albumin and protein concentrations (infection contrast: $P < 0.001$), but plasma urea and glucose concentrations were unaffected (infection contrast: $P \geq 0.32$). The plasma concentration of Arg was greater (infection contrast: $P = 0.003$) in infected compared with uninfected lambs, but the opposite was true for plasma Thr (infection contrast: $P = 0.005$). All other AA concentrations were not affected (infection contrast: $P \geq 0.12$) by infection.

The changes in PCV during the experiment are presented in Fig. 1. At the beginning of the experiment, PCV was the same in all treatments but started to differ in wk 2 between infected and uninfected lambs ($P < 0.001$). Regardless of the diets of infected lambs, PCV decreased ($P < 0.001$) steadily from wk 1 to 4 and

**Table 4.** Effect of cultivar, condensed tannins (CT), and infection with *Haemonchus contortus* on blood variables ($n = 6$)

| | Treatments[1] | | | | | | | *P*-values of contrasts[2] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item | Vi | Vi-PEG | Vu | Pi | Pi-PEG | Pu | SEM | Cultivar | CT | Infection |
| Plasma urea, mmol/L | 9.98 | 11.05 | 9.46 | 8.85 | 10.54 | 9.33 | 0.246 | 0.007 | <0.001 | 0.94 |
| Plasma glucose, mmol/L | 3.87 | 3.91 | 3.70 | 3.85 | 4.17 | 3.78 | 0.115 | 0.26 | 0.19 | 0.32 |
| Serum albumin, g/L | 33.5 | 33.2 | 38.5 | 32.6 | 32.4 | 39.6 | 0.57 | 0.64 | 0.64 | <0.001 |
| Serum protein, g/L | 51.4 | 49.7 | 58.6 | 48.8 | 48.7 | 58.7 | 0.96 | 0.13 | 0.37 | <0.001 |
| Plasma EAA,[3] μ*M* | | | | | | | | | | |
| Total EAA | 1221 | 989 | 1158 | 1217 | 1007 | 1280 | 60.0 | 0.36 | <0.001 | 0.98 |
| Arg | 202.9 | 158.9 | 151.9 | 171.8 | 170.6 | 148.6 | 11.52 | 0.43 | 0.06 | 0.003 |
| Cys | 15.6 | 16.3 | 16.5 | 17.6 | 15.3 | 18.7 | 1.92 | 0.50 | 0.69 | 0.59 |
| His | 64.7 | 41.0 | 46.3 | 45.8 | 44.3 | 47.6 | 4.58 | 0.21 | 0.01 | 0.08 |
| Leu | 129.0 | 103.3 | 119.0 | 130.1 | 95.7 | 132.4 | 8.81 | 0.75 | 0.002 | 0.66 |
| Ile | 99.3 | 73.6 | 81.0 | 122.7 | 99.6 | 118.8 | 24.36 | 0.16 | 0.33 | 0.65 |
| Lys | 183.8 | 125.3 | 165.4 | 164.9 | 131.6 | 153.6 | 10.80 | 0.36 | 0.002 | 0.18 |
| Met | 36.6 | 31.3 | 37.1 | 34.3 | 34.6 | 39.9 | 1.88 | 0.41 | 0.19 | 0.12 |
| Phe | 54.1 | 51.7 | 50.2 | 52.7 | 46.1 | 57.9 | 3.00 | 0.92 | 0.15 | 0.82 |
| Thr | 116.3 | 106.0 | 161.4 | 131.3 | 96.9 | 179.4 | 15.46 | 0.53 | 0.16 | 0.005 |
| Trp | 32.5 | 32.8 | 33.6 | 28.9 | 29.4 | 36.3 | 2.87 | 0.14 | 0.42 | 0.80 |
| Tyr | 81.9 | 68.1 | 67.1 | 85.0 | 80.2 | 94.0 | 11.42 | 0.55 | 0.89 | 0.15 |
| Val | 243.0 | 197.0 | 248.6 | 256.3 | 184.8 | 277.3 | 10.22 | 0.25 | <0.001 | 0.20 |
| Plasma NEAA,[4] μ*M* | | | | | | | | | | |
| Total NEAA | 1064 | 910 | 1056 | 980 | 1000 | 1066 | 96.5 | 0.92 | 0.24 | 0.49 |
| Ala | 157.2 | 108.0 | 146.2 | 147.6 | 142.6 | 159.9 | 13.02 | 0.24 | 0.05 | 0.96 |
| Asn | 42.8 | 34.5 | 40.6 | 35.1 | 37.1 | 39.0 | 3.70 | 0.45 | 0.40 | 0.81 |
| Asp | 10.2 | 12.4 | 10.3 | 11.9 | 9.8 | 11.3 | 1.28 | 0.99 | 0.97 | 0.86 |
| Gln | 252.0 | 232.4 | 281.5 | 239.3 | 250.7 | 240.7 | 16.36 | 0.39 | 0.81 | 0.35 |
| Gly | 290.0 | 249.8 | 291.7 | 259.2 | 286.3 | 299.9 | 21.29 | 0.80 | 0.75 | 0.33 |
| Pro | 102.8 | 120.0 | 96.9 | 114.0 | 95.1 | 129.9 | 11.22 | 0.49 | 0.94 | 0.66 |
| Ser | 66.1 | 53.9 | 64.4 | 60.6 | 72.0 | 66.8 | 6.07 | 0.32 | 0.95 | 0.32 |
| EAA:NEAA | 1.24 | 1.19 | 1.17 | 1.32 | 1.07 | 1.27 | 0.081 | 0.55 | 0.07 | 0.55 |

[1]Vi = infected lambs fed Visnovsky; Vi-PEG = infected lambs fed Visnovsky treated with polyethylene glycol; Vu = uninfected lambs fed Visnovsky; Pi = infected lambs fed Perly; Pi-PEG = infected lambs fed Perly treated with polyethylene glycol; Pu = uninfected lambs fed Perly.

[2]Cultivar = Vi, Vi-PEG, Vu vs. Pi, Pi-PEG, Pu; CT = Vi, Pi vs. Vi-PEG, Pi-PEG; Infection = Vi, Pi vs. Vu, Pu.

[3]EAA = essential and semiessential AA.

[4]NEAA = non-essential AA.

stabilized from then on in the infected lambs, whereas PCV remained stable in the uninfected lambs from the beginning. From wk 4 to 5, which marked the start of sainfoin feeding and also the administration of iron-dextran, PCV increased ($P < 0.001$) in both infected and uninfected lambs and then remained stable until wk 8. No differences in PCV were observed ($P = 0.99$) within treatments with infected ($P = 0.99$) or with uninfected lambs ($P = 0.99$). A development similar to PCV was observed for HB (data not shown). In contrast, MCV and MCHC did not differ ($P = 0.13$ and $P = 0.24$, respectively; data not shown) between infected and uninfected lambs. Lambs did not show any clinical signs of illness due to nematode infection.

### *Fecal Egg Counts and Adult Worm Burdens*

In the uninfected lambs, no *H. contortus* eggs were found in the feces throughout the entire experiment. Eggs started to appear in the feces of infected lambs during wk 3 postinfection and peaked in wk 4 postinfection (average of all 4 treatment groups: 33,000 eggs/g of fecal DM; Fig. 2). There were no differences ($P = 0.06$) in FEC between the 4 infected treatment groups from wk 2 to 4 postinfection. From wk 5, when sainfoin feeding started, FEC decreased ($P < 0.001$) in all 4 infected treatment groups. Differences ($P = 0.05$) between Vi and Pi occurred only in wk 5, whereas Vi-PEG and Pi-PEG differed ($P = 0.05$) only at wk 8. Furthermore, FEC was less ($P \leq 0$ 0.01) in Vi than in Vi-PEG in wk 7 and 8; similarly, FEC was also less ($P \leq 0.03$) in Pi than in Pi-PEG in wk 6 through wk 8. At the end of the experiment, a 53% decrease in FEC for Vi compared with Vi-PEG and a 48% decrease for Pi compared with Pi-PEG was observed. No differences between treatments ($P \geq 0.17$) were found in the worm burdens of the infected lambs (Table 5). Per capita female fecundity did not differ ($P = 0.27$) between Vi and Pi; however, it was less for

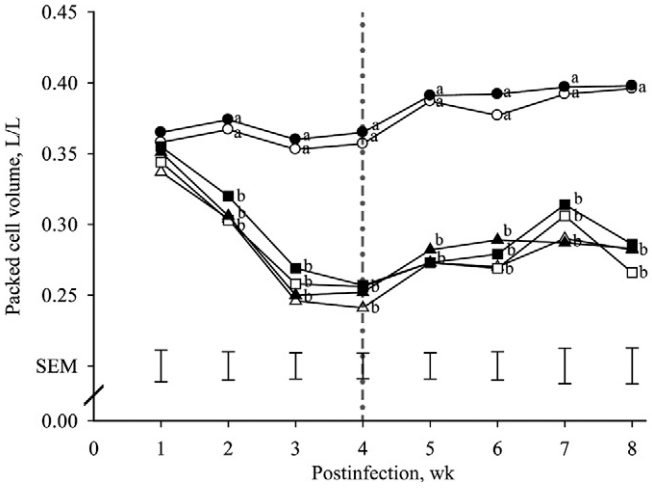


**Figure 1.** Packed cell volume over the experimental period of 8 wk. Open triangles, Visnovsky fed to infected lambs; open squares, Visnovsky supplemented with polyethylene glycol (PEG) and fed to infected lambs; open circles, Visnovsky fed to uninfected lambs; closed triangles, Perly fed to infected lambs; closed squares, Perly supplemented with PEG and fed to infected animals; filled circles, Perly fed to uninfected animals. Vertical bars represent SEM of the respective week postinfection. Within a week, means with different letters are significantly different at $P \leq 0.05$. The vertical dashed and dotted line indicates the beginning of feeding of the experimental forages.

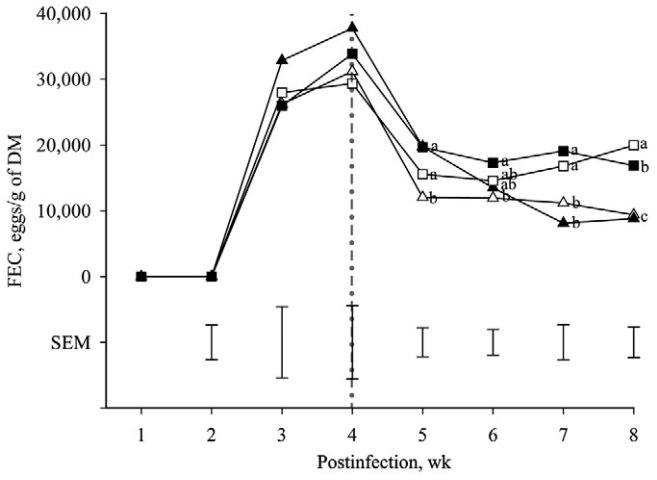


**Figure 2.** Fecal egg count (FEC) in infected lambs fed different forages beginning with the first egg excretion after 2 wk postinfection. Open triangle, Visnovsky fed to infected lambs; open square, Visnovsky supplemented polyethylene glycol (PEG) and fed to infected animals; filled triangle, Perly fed to infected lambs; filled square, Perly supplemented with PEG and fed to infected animals. Vertical bars represent SEM of the respective week postinfection. Within a week, means with different letters are significantly different at $P < 0.05$. The vertical dashed and dotted line indicates the beginning of feeding of the experimental forages.

Vi compared with Vi-PEG ($P = 0.008$), for Pi compared with Pi-PEG ($P = 0.03$), and for Pi-PEG compared with Vi-PEG ($P = 0.02$). No relationship (–0.01, $P = 0.99$) was found between intake of PD and FEC or adult worm counts (–0.16, $P = 0.61$). Similarly, CT intake did not correlate (0.21, $P = 0.51$) with FEC and (0.36, $P = 0.25$) with adult worm counts.

## DISCUSSION

Experimental infection with *H. contortus* was successful as shown by the FEC and abomasal worm counts. As expected, this changed the hematological status, serum albumin, and protein concentrations in response to the blood-sucking parasite (Scharenberg et al., 2008). This also resulted in certain, but limited, shifts in the plasma AA profile. The pathogenic impact of ovine hemonchosis can be markedly affected by protein supply from the diet (Abbott et al., 1986) and legumes such as sainfoin with high protein contents may meet the protein requirements of healthy lambs, even if CP availability is decreased because of CT. However, this is not necessarily the case for infected lambs (Scharenberg et al., 2008). *Haemonchus*-infection characteristically reduced body N retention due to increased urinary N losses (Rowe et al., 1988) and this was also observed in the present study. The greater urinary N excretion reflects the increased metabolic demands of infection and results in the spent protein being excreted via urine. Rowe et al. (1988) reported increased recycling of urea to the gastrointestinal tract, more urea synthesis from ammonia, but also more urea excretion in infected lambs compared with their uninfected pair-fed controls on a diet of alfalfa, a CT-free forage. The lambs in the present study received protein-rich (>19.0%) diets, and this possibly accounted for the generally mild nature of the infection. This is evident from the decreased, but still positive, ADG of infected lambs, and also in the biochemical and hematological values, which albeit decreased, were within the normal ranges. This might be partly a result of the iron-dextran administrated during wk 5 because of rapidly falling PCV; this may have

**Table 5.** Effect of cultivar and condensed tannins (CT) on total adult worm counts and per capita worm fecundity of infected lambs ($n = 6$; means and 95% confidence interval maximum likelihood)

| Item | Treatments[1] | | | | *P*-values | | | |
|---|---|---|---|---|---|---|---|---|
| | Vi | Vi-PEG | Pi | Pi-PEG | Vi vs. Pi | Vi vs. Vi-PEG | Pi vs. Pi-PEG | Vi-PEG vs. Pi-PEG |
| Mean adult worm counts | 2297 | 2378 | 2703 | 2972 | 0.20 | 0.81 | 0.52 | 0.17 |
| 95% confidence intervals | (2008 to 2642) | (1881 to 3067) | (2207 to 3358) | (2453 to 3647) | | | | |
| Mean per capita fecundity | 8.5 | 21.2 | 7.8 | 12.3 | 0.27 | 0.008 | 0.03 | 0.02 |
| 95% confidence intervals | (5.13 to11.76) | (13.81 to 28.4) | (4.74 to 10.69) | (8.84 to 15.75) | | | | |

[1]Vi = infected lambs fed Visnovsky; Vi-PEG = infected lambs fed Visnovsky treated with polyethylene glycol; Vu = uninfected lambs fed Visnovsky; Pi = infected lambs fed Perly; Pi-PEG = infected lambs fed Perly treated with polyethylene glycol; Pu = uninfected lambs fed Perly.

helped to stabilize PCV in infected lambs by restoring their depleted iron reserve pools. A similar response pattern in blood loss (maximal between 3 and 5 wk postinfection) and an increase in PCV as a result of iron-dextran administration in infected lambs was reported by Vegors (1973).

### *Nutritional and Anthelmintic Effects of the Different Sainfoin Cultivars*

The main objective of the present study was to determine whether the 2 sainfoin cultivars with different CT properties caused differing responses in the nutritional and parasitological traits of infected lambs. Visnovsky and Perly had been selected as test cultivars because they had different CT contents (4.7 vs. 5.9% in DM, respectively; Azuhnwi et al., 2011) and CT compositions (Azuhnwi et al., 2013). Consistent with that, the Visnovsky sward had a less CT concentration compared with the Perly sward (8.1 vs. 9.7% of DM). Thus, the differences in CT content were even larger. However, in the present study, both cultivars had much more CT, which by far exceeded the threshold of 4.5 to 5.5% of DM, where antiparasitic activity was expected (Min and Hart, 2003), and this likely leveled out cultivar differences. Despite the impure nature of the swards, relatively high CT contents were achieved compared with a previous study (Azuhnwi et al., 2011), which had been performed using almost pure sainfoin material and had been obtained from the second year of establishment in contrast to the first year of establishment in the present study. In contrast, Scharenberg et al. (2008) reported a halving of CT concentration from first to second year of harvest for the Visnovsky cultivar. Concerning CT properties, the Visnovsky material had more PD and a greater mDP than Perly as had been found earlier (Azuhnwi et al., 2013). A greater proportion of PD was expected to increase the biological activity of the CT (Theodoridou et al., 2011). This is based on the assertion that PD with 3 OH groups in the B-rings, compared with the 2 OH groups of PC, predisposes them to bind more strongly with macromolecules (Brunet and Hoste, 2006). In addition, a greater mDP (i.e., larger tannin polymers) may increase the astringency and thus the complex-forming ability of CT with proteins and carbohydrates (Aerts et al., 1999). However, in the present study, the nutritional response to the differences in CT composition and content were rather low. Cultivar effects were just observed on both ADF and NDF digestibility and plasma urea concentration. However, despite the greater % PD of Visnovsky CTs, both traits were decreased in Perly-fed lambs instead, which suggests that CT concentration rather than CT composition was responsible for the effects on both NDF and ADF digestibility and also urea concentration. Greater CT concentrations in forages are known to reduce fiber digestibility in sheep (Reed et al., 1990). Also, the effect on N balance was much more pronounced when feeding sainfoin with about 7.4% CT (Scharenberg et al., 2007) compared with sainfoin with about 3.6% CT (Scharenberg et al., 2008). Similar to the lack of a nutritional response to intercultivar differences, there was an almost complete absence in the response of parasitological and pathophysiological traits. A greater PD value was reported to be associated with increased antiparasitic activity in vitro (Brunet and Hoste, 2006; Hoste et al., 2006). However, this could not be confirmed by the present study, where no correlation could be found between PD intake and FEC or adult worm count. This highlights the difficulties inherent in extrapolating results from in vitro to in vivo experiments.

### *Nutritional and Anthelmintic Effects of Condensed Tannins*

We used PEG to evaluate the biological effect of CT in the infected lambs as this compound binds preferentially with CT. The PEG thus prevents the formation of complexes between CT- and other macromolecules, such as protein, fiber, and starch (Jones and Mangan, 1977). In doing so, it does not appear to affect digestion and can be added to the feed of ruminants as a means to eliminate CT effects (Priolo et al., 2000). In the present study, the classical adverse CT effect on the apparent digestibility of all nutrients was evident. Strong CT effects were also found by Scharenberg et al. (2007), who used the Visnovsky cultivar with a comparable CT content (7.4%). As expected, sainfoin CT redirected about 20% of urinary N to feces as is typical of CT containing forages (Mueller-Harvey, 2006). Incomplete dissociation of CT-protein complexes in the duodenum or renewed complex formation at the greater pH of the distal part of the small intestine (Jones and Mangan, 1977) may have enhanced endogenous N excretion (Kariuki and Norton, 2008), which could account for the greater fecal N. The end result of this is that N is excreted in a less environmentally polluting form, for fecal N outputs are converted to ammonia at a much slower rate and therefore retained in the soil, thus contributing to accumulation of soil OM, whereas urinary N is more rapidly volatilized to nitrous oxides which are potent greenhouse gasses (Waghorn, 2008). However, these findings appear to be in contrast to the greater plasma concentrations of several essential and semiessential AA. Consistent with that, plasma AA were increased by CT despite the reductions in apparent N digestibility in sheep fed the CT-containing *Lotus corniculatus* compared with their PEG controls (Waghorn et al., 1987). As there was an opposite response in body N retention,

either there were still 1 or more AA limiting body tissue formation, or the absolute level of metabolic AA supply was actually reduced due to the greater fecal N losses described above. Furthermore, rumen microbial protein synthesis might have been hampered by the CT because of the reduced amount of fermented OM. The literature on N retention in sainfoin-fed lambs is not consistent. A positive effect on N retention was reported by Aufrère et al. (2008), whereas no effects (Scharenberg et al., 2007, 2008; Theodoridou et al., 2010) have also been reported. Egan and Ulyatt (1980) pointed out that apparent N digestibility is an inadequate index for predicting the likely N retention; this is especially the case with sainfoin, where increased recycling and degradation of urea was the only major N variable associated with N retention. Our results suggest that urea recycling was improved by sainfoin CT because both plasma and urinary urea concentrations were decreased by CT, which is in agreement with Scharenberg et al. (2007).

There were clear CT effects on FEC and per capita female fecundity in the present study. This was in line with other studies which reported inhibitory effects of sainfoin extracts on larval exsheathment, as well as on larval migration and motility of adult worms in vitro (Paolini et al., 2004; Brunet et al., 2008), and a decrease in FEC and worm fecundity in infected sheep consuming sainfoin hay and silage (Heckendorn et al., 2006, 2007; Manolaraki et al., 2010). Likewise, the inhibitory effect of PEG on CT activities has been shown in vitro (Paolini et al., 2004; Brunet et al., 2008), but its effectiveness in infected ruminants is not yet clear. Kabasa et al. (2000) confirmed that PEG deactivated browse CT in free-ranging goats, whereas Niezen et al. (1998) and Hoste et al. (2006) questioned its effectiveness. Condensed tannins can influence key biological processes (e.g., they can inhibit enzymatic activity in adult and larval *Haemonchus*; Hoste et al., 2006). However, the work of Heckendorn et al. (2006) appears to be the only study that reported a significant reduction (50%) in adult *Haemonchus* populations in experimentally infected sheep that were fed sainfoin hay and silage compared with a CT-free control. Hoste et al. (2006) reported that when incoming larvae encounter tannins in the digestive tract, the main effect is on larval establishment and, consequently, worm numbers. However, when CT are administered after establishment of the parasites, egg production of female worms is affected. Therefore, a reduction in FEC may result from either decreased worm numbers or reduced female worm fecundity, or both; however, the fecundity seems to be the main CT effect (Heckendorn et al., 2007; Manolaraki et al., 2010). Furthermore, our results confirm the findings of Manolaraki et al. (2010) that the decrease in FEC, which resulted from sainfoin CT, was not accompanied by changes in pathophysiological traits. This implies that the most important effect of sainfoin CT is to lower the number of *Haemonchus* worm eggs that are shed on the pasture and this in turn will reduce pasture infectivity.

### *Antiparasitic Effects of Sainfoin not Mediated by Condensed Tannins*

A decline in FEC was observed in all treatment groups immediately after the onset of sainfoin feeding. The proportion of infective larvae which were finally recovered as adults ranged between 40 and 50% across all 4 infected treatments. This is in contrast with a parallel sheep experiment using the same *Haemonchus* isolate and larval dose, where a greater percentage of larvae were recovered as adults (67%) in untreated animals (H. Hertzberg, personal communication). This indicates that in the present experiment the number of adult *Haemonchus* worms may have been reduced independent of PEG feeding. Indeed, Hoste et al. (2006) suggested that other phenolic compounds, which are not bound by PEG, can also have an antiparasitic effect, and this could explain the lower FEC in all infected lambs. In fact, in vitro studies with sainfoin extracts by Barrau et al. (2005), who used the larval migration inhibition assay, showed that other polyphenols, such as rutin, nicotiflorin, and narcissin (all flavonol glycosides), also inhibited larval migration. Furthermore, Regos et al. (2009) found that rutin and catechin were of the most abundant phenolic compounds in sainfoin, and this lends further credence to the above argument.

### *Conclusions*

In conclusion, the absence of substantial cultivar effects on most digestive, metabolic, and parasitological variables suggests that either the differences in CT content and CT composition were too small to cause noticeable responses, or have acted in a compensatory way. Where differences in responses existed between cultivars, CT concentration, rather than CT composition, seem to better explain them. Further studies will need to establish whether cultivars with more contrasting CT properties may differ sufficiently so that cultivars can be selected for future sainfoin improvement programs. Such studies, as well as dose–response approaches, should also concentrate on the determination of the optimum level of CT that could enhance bypass proteins and reduce the concentration of FEC. The differences in FEC between infected lambs fed with and without PEG suggests that sainfoin CT can mitigate pasture infectivity. However, the reduction was too low to expect any significant benefits in an *Haemonchus*-dominated system. Therefore, the use of sainfoin CT for controlling *H. contortus* should only be 1 component within an integrated worm control system.

## LITERATURE CITED


Abbott, E. M., J. J. Parkins, and P. H. Holmes. 1986. The effect of dietary protein on the pathogenesis of acute ovine haemonchosis. Vet. Parasitol. 20:275–289.

Aerts, R. J., T. N. Barry, and W. C. McNabb. 1999. Polyphenols and agriculture: Beneficial effects of proanthocyanidins in forages. Agric. Ecosyst. Environ. 75:1–12.

Agroscope Liebefeld-Posieux. 2010. Fütterungsempfehlungen und nährwerttabellen für wiederkäuer (Feeding recommendations and nutrient tables). Forschungsanstalt Agroscope, Liebefeld-Posieux, Switzerland. Online ed. www.alp.admin.ch/dokumentation/00611/00631/index.html. (Accessed November 2010).

AOAC. 1995. Official Methods of Analysis, 16th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Aufrère, J., M. Dudilieu, and C. Poncet. 2008. *In vivo* and *in situ* measurements of the digestive characteristics of sainfoin in comparison with lucerne fed to sheep as fresh forages at two growth stages and as hay. Animal 2:1331–1339.

Azuhnwi, B. N., B. Boller ,F. Dohme-Meier, H. D. Hess, M. Kreuzer, E. Stringano, and I. Mueller-Harvey. 2013. Exploring variation in proanthocyanidin composition and content of sainfoin (*Onobrychis viciifolia*) J. Sci. Food. Agric. DOI 10.1002/jsfa.6119.

Azuhnwi, B. N., B. Boller, M. Martens, F. Dohme-Meier, S. Ampuero, S. Günter, M. Kreuzer, and H. D. Hess. 2011. Morphology, tannin concentration and forage value of 15 Swiss accessions of sainfoin (*Onobrychis viciifolia* Scop.) as influenced by harvest time and cultivation site. Grass Forage Sci. 66:474–487.

Barrau, E., N. Fabre, I. Fouraste, and E. Hoste. 2005. Effect of bioactive compounds from sainfoin (*Onobrychis viciifolia* Scop.) on the in vitro larval migration of *Haemonchus contortus*: Role of tannins and flavonol glycosides. Parasitology 131:531–538.

Brunet, S., F. Jackson, and H. Hoste. 2008. Effects of sainfoin (*Onobrychis viciifolia*) extract and monomers of condensed tannins on the association of abomasal nematode larvae with fundic explants. Int. J. Parasitol. 38:783–790.

Brunet, S., and H. Hoste. 2006. Monomers of condensed tannins affect the larval exsheathment of infective nematodes of ruminants. J. Agric. Food Chem. 54:7481–7487.

Egan, A. R., and M. J. Ulyatt. 1980. Quantitative digestion of fresh herbage by sheep: VI. Utilization of nitrogen in five herbages. J. Agric. Sci. 94:47–56.

European Union. 1998. Commission Directive 98/64/EC establishing community methods of analysis for the determination of amino acids, crude oils and fats, and olaquindox in feedstuffs and amending Directive 71/393/EEC. Off. J. Eur. Comm. L257, Part A:1428.

Gea, A., E. Stringano, R. H. Brown, and I. Mueller-Harvey. 2011. *In situ* analysis and structural elucidation of sainfoin (*Onobrychis viciifolia*) tannins for high throughput germplasm screening. J. Agric. Food Chem. 59:495–503.

Heckendorn, F., D. A. Häring, V. Maurer, M. Senn, and H. Hertzberg. 2007. Individual administration of three tanniferous forage plants to lambs artificially infected with *Haemonchus contortus* and *Cooperia curticei*. Vet. Parasitol. 146:123–134.

Heckendorn, F., D. A. Häring, V. Maurer, J. Zinsstag, W. Langhans, and H. Hertzberg. 2006. Effect of sainfoin (*Onobrychis viciifolia*) silage and hay on established populations of *Haemonchus contortus* in lambs. Vet. Parasitol. 142:293–300.

Hoste, H., F. Jackson, S. Athanasiadou, S. M. Thamsborg, and S. O. Hoskin. 2006. The effects of tannin-rich plants on parasitic nematodes in ruminants. Trends Parasitol. 22:253–261.

Jones, W. T., and J. L. Mangan. 1977. Complexes of the condensed tannins of sainfoin (*Onobrychis viciifolia*) with fraction 1 leaf protein and with submaxillary mycoprotein, and their reversal by polyethylene glycol and pH. J. Sci. Food Agric. 28:126–136.

Kabasa, J. D., J. Opuda-Asibo, and U. ter Meulen. 2000. The effect of oral administration of polyethylene glycol on fecal helminth egg counts in pregnant goats grazed on browse containing condensed tannins. Trop. Anim. Health Prod. 32:73–86.

Kariuki, I. W., and B. W. Norton. 2008. The digestion of dietary protein bound by condensed tannins in gastro-intestinal tract of sheep. Anim. Feed Sci. Technol. 142:197–209.

Manolaraki, F., S. Sotiraki, A. Stefanakis, V. Skampardonis, M. Volanis, and H. Hoste. 2010. Anthelmintic activity of some Mediterranean browse plants against parasitic nematodes. Parasitology 137:685–696.

Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feed with refluxing beakers or crucibles: Collaborative study. J. AOAC Int. 85:1217–1240.

Min, B. R., T. N. Barry, G. T. Attwood, and W. C. McNabb. 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: A review. Anim. Feed Sci. Technol. 106:3–19.

Min, B. R., and S. P. Hart. 2003. Tannins for suppression of internal parasites. J. Anim. Sci. 81:E102–E109.

Mueller-Harvey, I. 2006. Unraveling the conundrum of tannins in animal nutrition and health. J. Sci. Food Agric. 86:2010–2037.

Niezen, J. H., G. C. Waghorn, and W. A. G. Charleston. 1998. Establishment and fecundity of *Ostertagia circumcincta* and *Trichostrongylus colubriformis* in lambs fed lotus (*Lotus pedunculatus*) or perennial ryegrass (*Lolium perenne*). Vet. Parasitol. 78:13–21.

Paolini, V., I. Fouraste, and H. Hoste. 2004. *In vitro* effects of three woody plant and sainfoin extracts on third-stage larvae and adult worms of three gastrointestinal nematodes. Parasitology 129:69–77.

Priolo, A., G. C. Waghorn, M. Lanza, L. Biondi, and P. Pennisi. 2000. Polyethylene glycol as a means for reducing the impact of condensed tannins in carob pulp: Effects on lamb growth performance and meat quality. J. Anim. Sci. 78:810–816.

R Development Core Team. 2011. R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. www.R-project.org (accessed Oct. 20, 2011).

Reed, J. D., H. Soller, and A. Woodward. 1990. Fodder tree and straw diets for sheep: Intake, growth, digestibility, and the effects of phenolics on nitrogen utilization. Anim. Feed Sci. Technol. 30:39–50.

Regos, I., A. Urbanella, and D. Treutter. 2009. Identification and quantification of phenolic compounds from the forage legume sainfoin (*Onobrychis viciifolia*). J. Agric. Food Chem. 57:5843–5852.

Rowe, J. B., J. V. Nolan, G. Dechaneet, E. Teleni, and P. H. Holmes. 1988. The effect of Haemonchosis and blood-losses into the abomasum on digestion in sheep. Br. J. Nutr. 59:125–139.

Scharenberg, A., Y. Arrigo, U. Wyss, H. D. Hess, M. Kreuzer, and F. Dohme. 2007. Effect of feeding dehydrated and ensiled tanniferous sainfoin (*Onobrychis viciifolia*) on nitrogen and mineral digestion and metabolism of lambs. Arch. Anim. Nutr. 61:390–405.

Scharenberg, A., F. Heckendorn, Y. Arrigo, H. Hertzberg, A. Gutzwiller, H. D. Hess, M. Kreuzer, and F. Dohme. 2008. Nitrogen and mineral balance of lambs artificially infected with *Haemonchus contortus* and fed tanniferous sainfoin (*Onobrychis viciifolia*). J. Anim. Sci. 86:1879–1890.

Stewart, J. L., F. Mould, and I. Mueller-Harvey. 2000. The effect of drying treatment on the fodder quality and tannin content of two provenances of *Calliandra calothyrsus* Meissner. J. Sci. Food Agric. 80:1461–1468.

Terrill, T. H., A. M. Rowan, G. B. Douglas, and T. N. Barry. 1992. Determination of extractable and bound condensed tannin concentrations in forage plants, protein-concentrate meals and cereal-grains. J. Sci. Food Agric. 58:321–329.

Theodoridou, K., J. Aufrère, D. Andueza, J. Pourrat, A. Le Morvan, E. Stringano, I. Mueller-Harvey, and R. Baumont. 2010. Effects of condensed tannins in fresh sainfoin (*Onobrychis viciifolia*) on *in vivo* and *in situ* digestion in sheep. Anim. Feed Sci. Technol. 160:23–38.

Theodoridou, K., J. Aufrère, V. Niderkorn, D. Andueza, A. Le Morvan, F. Picard, and R. Baumont. 2011. *In vitro* study of the effects of condensed tannins in sainfoin on the digestive process in the rumen at two vegetative cycles. Anim. Feed Sci. Technol. 170:147–159.

Torgerson, P. R., M. Schnyder, and H. Hertzberg. 2005. Detection of anthelmintic resistance: A comparison of mathematical techniques. Vet. Parasitol. 128:291–298.

Vegors, H. H. 1973. Effect of intramuscular injections of iron-dextran on ovine Haemonchosis. P. Helm. Soc. Wash. 40:66–68.

Waghorn, G. 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production—Progress and challenges. Anim. Feed Sci. Technol. 147:116–139.

Waghorn, G. C., M. J. Ulyatt, A. John, and M. T. Fisher. 1987. The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed on *Lotus corniculatus* L. Br. J. Nutr. 57:115–126.