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Tanniferous forage plants: Agronomic performance, palatability and efficacy against parasitic nematodes in sheep

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Abstract

Tanniferous forage plants can have beneficial effects on ruminant productivity and health (improved protein supply, bloat safety and antiparasitic properties). However, condensed tannins can also lower palatability, voluntary feed intake and digestibility. The aim of our interdisciplinary project was to generate basic knowledge on plant management, feed palatability and the antiparasitic properties of tanniferous forage plants for their practical application in agronomy, focusing on their usefulness in controlling gastrointestinal nematodes in organic farming. We found that *Onobrychis viciifolia* (sainfoin), *Lotus corniculatus* (birdsfoot trefoil) and *Cichorium intybus* (chicory) were suitable for cultivation under the given temperate climatic conditions, whereas *Lotus pedunculatus* (big trefoil) was soon outcompeted by unsown species. Growing the tanniferous plant species in a mixture with *Festuca pratensis* (meadow fescue) rather than in a monoculture had the advantage of increasing total dry matter (DM) yield (especially in the case of tanniferous legumes) and of reducing the DM proportions of unsown species. However, due to dilution by non-tanniferous *F. pratensis*, the tannin concentrations of mixtures were clearly lower and the seasonal fluctuations in tannin concentrations greater than that of monocultures. Across species, tannin concentrations were highest for *O. viciifolia*, followed by *L. corniculatus* and very low for *C. intybus*. Palatability of all tanniferous forages was comparable to that of a ryegrass/clover mixture when fed as dried forage and, when offered as silage, palatability of *O. viciifolia* was clearly superior to that of the respective ryegrass/clover control. Administration of dried or ensiled *O. viciifolia* reduced parasite egg counts in feces of lambs co-infected with the gastrointestinal nematode species *Haemonchus contortus* and *Cooperia curticei*. We conclude that *O. viciifolia* is the most promising among the tested tanniferous forage plant species due to its suitability for cultivation, its high tannin concentration, its high palatability and its antiparasitic activity even in dried or ensiled form.

Key words: condensed tannins, proanthocyanidins, forage cultivation, yield, silage, palatability, gastrointestinal nematodes, parasite control, sheep

Introduction

Recent experiments in agronomy and parasitology suggest that moderate dietary concentrations of condensed tannins can affect the health and performance of sheep and other small ruminants beneficially[1–5]. For example, condensed tannins were found to increase life weight gain, fecundity and wool production in sheep[6,7]. With regard to animal health, condensed tannins are known to diminish the risk of bloat, lower fecal worm egg excretion and reduce worm burdens of parasitized ruminants[3,5]. However, high concentrations of condensed tannins can have a negative impact on ruminants. They can lower palatability and digestibility[5,8] and, in extreme cases, they can lead to mucosal lesions[9].

In an interdisciplinary project involving research on plant cultivation, animal nutrition and parasitology, we addressed key questions concerning the implementation of tanniferous forage plants as an alternative control strategy against gastrointestinal nematodes in animal husbandry: Which tanniferous species and cultivars (cv.) perform well with regard to agronomic properties such as yield, persistence and competitive ability? What are their concentrations of condensed tannins and what are the most important drivers of fluctuations in tannin concentrations under field conditions? How palatable are different tanniferous forage plant species in comparison to a ryegrass/clover mixture? How effective is the most promising plant species against gastrointestinal nematodes in sheep? Can tanniferous forages be conserved without losing their desirable anthelmintic properties?

Plant Sciences and Forage Cultivation

The expressions 'condensed tannins' or synonymously 'proanthocyanidins' summarize a large and chemically diverse group of phenolic polymers with the ability to bind proteins and other macromolecules. Apart from their strong affinity for proteins, condensed tannins can form complexes with metal ions and have radical scavenging properties[10]. The structure of condensed tannins consists of a linkage of a series of monomers based on flavan-3-ol nuclei or derivatives thereof[11]. Variations in the structure of condensed tannins (and supposedly of their bioactivity) can occur through differences in the number of monomers, the positions between which these monomers are interlinked, the oxygenation pattern of the monomers or the stereochemistry of the polymer[11,12]. Condensed tannins exist as water-soluble oligomers and as insoluble polymers[10]. Tanniferous plants usually contain mixtures of differently structured tannins rather than one specific type of molecule[11,13]. The quantity and structural composition of tannins vary between different tissues and can change during plant development[13,14]. Condensed tannins occur predominantly in woody plants but can also be found in some herbaceous plant species, for example in representatives of the Rosaceae and Fabaceae families[11]. Within plants, condensed tannins are concentrated in cell vacuoles[12] and usually occur in higher concentrations in leaves and reproductive organs than in stems and roots[14].

The bioactivity of condensed tannins is thought to be a function of tannin concentrations defined as the ratio between consumed tannin and consumed protein or feed[11] rather than of the absolute amount of consumed tannin. As a rule of thumb it has repeatedly been suggested that tannin concentrations below $50\,g\,kg^{-1}$ DM produce positive effects in ruminants, while concentrations in excess of $50\,g\,kg^{-1}$ DM affect the animal negatively[1,4]. Today's experimental evidence relativizes this rule to the extent that tannins from different sources may vary in their 'potency'[5] and that it is likely that different thresholds need to be defined for different tannins and, thus, different forage plant species.

Genotypic and phenotypic variation of type and quantity of secondary metabolites have often been studied within the paradigm that their primary role is plant defensive and their effect on consumers detrimental. The accumulated experimental evidence for and against the so-called plant defense theory[15–18] is equivocal[19,20] and its usefulness for the practical application in agronomy disputable[14]. Previous studies of seasonal fluctuations of tannin concentrations in forage plants yielded conflicting results[14,21–24] and mechanisms driving seasonal and developmental dynamics of tannin concentrations under field conditions are not yet well understood. Based on an experiment with potted *Onobrychis viciifolia* and *Lotus corniculatus*, it has been suggested that fluctuations in condensed tannin concentrations in harvestable aboveground biomass are functions of biomass proportions between tannin-rich leaves and tannin-poor stems in pure stands, and of biomass proportions between tanniferous and non-tanniferous plant species in mixed stands[14]. At present, it is unclear to what extent these hypotheses hold true under field conditions.

For a successful implementation of a gastrointestinal nematode control strategy based on tanniferous forage plants, it is essential that the tanniferous forage crop provides a high yield, has a high competitive ability and an elevated but predictable concentration of condensed tannins. Especially under low input conditions, the agronomic performances of species mixtures have been reported to be superior to those of monocultures. In comparison to monocultures, mixtures are often characterized by higher dry matter (DM) yields[25], improved evenness of the seasonal growth patterns[26] and increased resistance against weed invasion[25]. Until now, comparative assessments of agronomic performances of promising tanniferous forage plants and concurrent investigations of tannin concentration dynamics under field conditions have been scarce.

Therefore we evaluated yield, resistance to weed invasion and tannin concentrations of 12 cultivars of four commercially available, tanniferous forage plant species which were either sown in a monoculture or in a mixture with the grass species *Festuca pratensis* in a three-year field experiment. We put special emphasis on investigating to what extent potential seasonal fluctuations of condensed tannin concentrations in the yield can be attributed to shifts in the relative DM contribution of tanniferous plants to total DM yield.

Animal Nutrition

Palatability designates the sum of all physical and chemical characteristics of a diet that evoke appetite, such as olfactory, gustatory and tactile stimuli during foraging and chewing[27]. Repeated feeding of a particular diet enables an animal to anticipate potential nutritional and physiological postingestive feedbacks and eventually to develop a preference for or an aversion against it[28]. The perception of a diet, and subsequent feed preferences, vary among individual animals according to differences in physiology and experience[29]. Therefore, palatability and food preference are questions of physiological traits of the animal, of individual experience and of the feed quality of the forage in question in relation to the range of feeds available to the animal.

There is a wealth of evidence suggesting that condensed tannins in plants influence the voluntary feed intake, palatability and digestibility of the fodder[2,4,5]. It is thought that the importance of condensed tannins in animal nutrition lies mainly in their ability to bind proteins. For example, the astringent taste of tanniferous plants is a consequence of the binding of tannins to salivary proteins. The lower true digestibility of feed proteins, the increase of the excretion of endogenous protein in pigs[30], and the lower ruminal protein degradability combined with a lower apparent digestibility in ruminants[31] are results of the binding of tannins to feed protein and endogenous proteins. While protein precipitation in the intestine lowers amino acid availability and affects the ruminant adversely, protein precipitation and protein protection from microbial degradation in the rumen can be desirable and beneficial to the animal[32]. The binding of condensed tannins to protein in the rumen (pH 6–7) lowers ammonia production and reduces the metabolic strain of the liver (2). Furthermore, it enhances the protein flow towards the small intestine and, provided the tannin–protein complexes dissociate in the more acid medium of the abomasum ($pH < 3$), raises the animal's supply of essential amino acids[5,33]. An improved protein supply of the ruminant is one of the possible pathways leading to an increased tolerance of ruminants to gastrointestinal parasite infections[3,34]. In addition to the likely impact of condensed tannins on nitrogen metabolism, tannins may also affect the mineral supply of the animal[35,36]. For the practical application of tanniferous forages in livestock production, e.g. as an alternative nematode control strategy, administration of forage plants in a conserved form (either as dried or ensiled forage) is of great interest. In previous experiments, condensed tannins were reported to depress proteolysis thereby reducing the loss of forage protein during the ensiling process[37]. Furthermore, it was observed that ensiling reduced tannin concentrations of sorghum[38]. Up to now, little is known about the effect of ensiling on the palatability of tanniferous forage plants.

Feeding trials provide an ideal means of obtaining a brief and summary-like answer to the complex question of whether or not a certain (tanniferous) forage plant species is suitable for its consumer, and allow an assessment of voluntary feed intake and possible side effects of the feed. In this study, we aimed to identify the most promising tannin-containing plant species and conservation method in terms of the wethers' acceptance and, thus, palatability of dried or ensiled *O. viciifolia*, *L. corniculatus* and *Cichorium intybus* in relation to a dried or ensiled non-tannin-containing ryegrass/clover mixture, respectively.

Parasitology

Infections with gastrointestinal nematodes represent a major constraint on the profitability of sheep, goat and cattle production. This is particularly pronounced in low-input farming where animals are grazing on pastures, in contrast to intensive production systems in which access to pasture is limited or even absent[39]. The organic farmer is confronted with the problem of (i) reducing the infection pressure to an acceptable level and (ii) having largely to resign himself to the use of conventional anthelmintic drugs in order to keep within the organic guidelines. As complementary approaches to control gastrointestinal parasitism of small ruminants, such as the exploitation of the genetic resistance of livestock[40], biological control of the free-living larval stages in the feces by means of nematophagous fungi[41,42], manipulation of grazing management[43] and dietary supplementation with protein[44], were each only partially effective, the control of endoparasites remains largely based on the application of synthetic anthelmintic drugs. The development of gastrointestinal parasite populations resistant against most of the currently available anthelmintics further highlights the need for alternative parasite control strategies[45].

Recent experiments with tanniferous forages administered to sheep and goats infected with gastrointestinal nematodes yielded promising results[3]. Tanniferous forages that were grazed, or freshly administered, to naturally or artificially infected animals often resulted in a decreased fecal egg count (i.e. the number of worm eggs per gram feces of the host), which was sometimes accompanied by a reduction of worm burden (i.e. the number of adult worms) or reductions in worm fecundity. The potential of condensed tannins as an antiparasitic agent has been substantiated by various *in vitro* and *in vivo* experiments: condensed tannins were found to reduce the parasites' motility, possibly by binding to surface proteins, to exert a toxic effect on parasite eggs, larvae or adult worms and to hinder larval development[3,46]. Alternatively or complementary to these 'direct effects', condensed tannins can exert indirect effects. For example, they may help to improve the host's immune response to parasitism (i.e. resistance) by enhancing the ruminant's supply of proteins and essential amino acids[3].

Administering tanniferous forages to parasitized ruminants in a conserved form, either as hay or silage, could potentially enhance the practicability and efficacy of the treatment. However, experiments with conserved forages remain few. Therefore, we tested the feeding of *O. viciifolia* hay and silage, respectively, to lambs co-infected with *Haemonchus contortus* and *Cooperia curticei* in comparison to non-tanniferous control feeds[47].

Methods

Agronomic performance of tanniferous forage plants

We assessed the yield, the competitive ability and the tannin concentration in the yield of 12 cultivars of four tanniferous plant species sown in monoculture or in a mixture with a non-tanniferous grass species. Plant performances were observed from sowing in spring 2004 until the last harvest in May 2006. The experiment included *O. viciifolia* (sainfoin, fam. Fabaceae: ecotype Alvaschein, commercial seed, cv. Visnovsky), *Lotus pedunculatus* (big trefoil, fam. Fabaceae: cv. Grasslands Maku, cv. Barsilvi, cv. Grasslands Sunrise), *L. corniculatus* (birdsfoot trefoil, fam. Fabaceae: cv. Odenwälder, cv. Lotar, cv. Oberhaunstädter), *Cichorium intybus* (chicory, fam. Asteraceae: cv. Grasslands Puna, cv. Forage Feast, cv. INIA Lacerta). For the mixtures, we selected *F. pratensis* (meadow fescue, fam. Poaceae: cv. Preval), a grass suitable for low-input farming with a high nutritive quality but with limited potential as a competitor.

The field experiment was done at Agroscope Reckenholz-Tänikon, Research Station ART, in Zurich, Switzerland (47°26′N, 8°30′E). The block experiment (three blocks) was sown with 'plant species' as the main plot factor, 'cultivar' as the subplot factor and 'purely sown versus mixture' as the sub-subplot factor[48]. Within each main plot (species), the cultivars were assigned randomly to pairs of sub-subplots with an area of $9\,m^2$ ($1.5\,m \times 6\,m$) each. On one of these plots the tanniferous cultivar was sown in monoculture; on its neighboring plot, it was sown in a mixture with the grass *F. pratensis*. To complete the design, each block contained two grass monocultures of $9\,m^2$ size (i.e. *F. pratensis* cv. Preval). Sowing densities of monocultures of *O. viciifolia*, *L. pedunculatus*, *L. corniculatus*, *C. intybus* and *F. pratensis* corresponded to 180, 18, 18, 4 and $25\,kg\,ha^{-1}$ of germinable seed, respectively. For the mixtures, we replaced 40% of the respective tanniferous plant species seed by 40% of *F. pratensis* seed (i.e. $25\,kg\,ha^{-1} \times 40\% = 10\,kg\,ha^{-1}$). Per block, one grass sward and all plots containing legumes (i.e. plants of the family Fabaceae with the ability of N_2-fixation by means of symbiotic rhizobia) were fertilized with only $25\,kg\,N\,ha^{-1}$ after each harvest. The remaining grass monocultures and the chicory plots were fertilized with $50\,kg\,N\,ha^{-1}$ after each harvest.

Swards were harvested twice in the year of sowing (i.e. 2004), four times in 2005 and a last time in spring 2006. On each of these occasions, three bands of 10 cm width were cut across every sward at 7 cm above ground level for an assessment of the botanical composition of the harvest and a quantification of its tannin concentration. A random subsample (one-third) of that plant material was immediately put on ice for transportation and dried at 60°C for 48 h as soon as possible, ground to pass through a 0.75 mm sieve, and stored in the dark at room temperature before it was analyzed for condensed tannins using a butanol–HCl-assay with a *L. pedunculatus* standard[49]. The remaining two-thirds of the plant material were separated into 'sown tanniferous species', 'sown *F. pratensis*' and 'unsown species'. The separated plant fractions were dried at 100°C for 24 h to calculate their relative contributions to the total DM yield. After collecting the botanical samples, swards were harvested by machine, 7 cm above ground level and the total DM yield of each sward was determined.

Feed palatability

In two consecutive but independent experiments of 20 days' duration each, the palatability for wethers of three tanniferous forage plant species (i.e. *O. viciifolia*, *L. corniculatus* and *C. intybus*) was tested in comparison to a control feed consisting of a ryegrass/clover mixture. In a first experiment, feeds were offered as dried forages; in a second experiment they were offered as silages.

O. viciifolia cv. Visnovsky, *L. corniculatus* cv. Oberhaunstädter, *C. intybus* cv. Grasslands Puna and a ryegrass/white clover/red clover mixture were grown in 2003 at Agroscope Liebefeld-Posieux, Research Station ALP, Posieux (46°46′N, 7°06′E), Switzerland. The tanniferous forages were topped once at the beginning of July, harvested in August and ensiled in 700-liter containers. Second harvests were taken at the end of September (*C. intybus*) or in mid October (*O. viciifolia, L. corniculatus*), respectively, and dehydrated at 30°C to a water content below 10% using a special drying system (Physitech, Wabern, Switzerland) that minimized the loss of tannin-rich plant leaf material. The non-tanniferous control mixture was taken from the fourth cut at the end of September from a ley and was likewise dried or ensiled. The tannin concentrations of the forages were analyzed using a butanol–HCl-assay with a *L. pedunculatus* standard[49].

The experiment involved three groups of adult Oxford wethers ($n = 6$) corresponding to the three tanniferous forage plant species. On average, wethers were 4.1 ± 1.8 years old and had a weight of 87.6 ± 7.2 kg; they were individually housed in pens. Wethers were offered a choice between the respective tanniferous forage and the control feed, which were presented simultaneously in two separate boxes. During the first 10 days of each experiment, the diets contained 110% of the maintenance energy requirement and were given in equal portions twice a day. The maintenance energy requirement [ME_m in MJ; equation (1)] was calculated as a function of the life weight (LW) of each animal and expressed as metabolizable energy[50]:

$$ME_m = 0.38 \times LW^{0.75} \quad (1)$$

During the second 10 days, the sheep received half of the experimental diets (55% ME_m) at 07:00 h and additionally low-quality hay (100% ME_m) at 16:00 h. Individual forage-specific feed intake was measured twice a day: once after a short evaluation period t ($t = 7.5$ min for ensiled forage and $t = 15$ min for dried forage) and once in the afternoon at

Table 1. Total yield, DM proportion of sown species, DM proportion of tanniferous species and tannin concentration of total yield harvested in the year 2005 (mean ± SE; $n = 3$, swards were cut four times in 2005). All cultivars were sown in 2004 either as pure stands or in mixture with *F. pratensis*. CT, condensed tannin.

	Total yield ($t\,ha^{-1}\,yr^{-1}$ DM)		Sown species (% of total yield)		Tanniferous species (% of total yield)		Tannin conc. of yield ($g\,CT\,kg^{-1}$ DM)	
	Pure	**Mixture**	**Pure**	**Mixture**	**Pure**	**Mixture**	**Pure**	**Mixture**
O. viciifolia [1]								
Alvaschein	11.0 ± 0.3	16.4 ± 1.1	46 ± 8	90 ± 2	46 ± 8	6 ± 1	21 ± 4	4 ± 1
Commercial seed	11.1 ± 0.2	16.4 ± 1.1	38 ± 13	96 ± 1	38 ± 13	6 ± 3	17 ± 3	4 ± 2
Visnovsky	13.0 ± 1.2	16.5 ± 1.6	76 ± 6	98 ± 1	76 ± 6	36 ± 3	24 ± 2	12 ± 1
L. pedunculatus [1]								
Maku	7.7 ± 0.3	18.1 ± 1.0	19 ± 3	88 ± 1	19 ± 3	5 ± 2	13 ± 3	4 ± 1
Barsilvi	9.0 ± 1.1	17.1 ± 0.1	2 ± 1	76 ± 1	2 ± 1	1 ± 1	4 ± 1	1 ± 1
Sunrise	8.6 ± 1.1	17.6 ± 1.0	5 ± 1	83 ± 3	5 ± 2	1 ± 1	5 ± 1	2 ± 1
L. corniculatus [1]								
Odenwälder	9.9 ± 1.0	18.0 ± 1.2	70 ± 12	95 ± 3	70 ± 12	21 ± 9	17 ± 1	6 ± 1
Lotar	11.0 ± 1.0	18.4 ± 1.1	77 ± 5	97 ± 1	77 ± 5	32 ± 9	18 ± 2	10 ± 2
Oberhaunstädter	10.0 ± 1.5	17.3 ± 1.1	77 ± 6	89 ± 4	77 ± 6	39 ± 10	15 ± 1	8 ± 1
C. intybus [2]								
Puna	12.8 ± 1.0	12.8 ± 0.6	100 ± 0	100 ± 1	100 ± 1	94 ± 2	5 ± 1	4 ± 1
Forage Feast	9.9 ± 0.5	12.6 ± 0.7	99 ± 1	98 ± 2	99 ± 1	72 ± 9	5 ± 1	3 ± 1
Lacerta	12.1 ± 1.2	12.5 ± 1.5	100 ± 0	100 ± 1	100 ± 1	87 ± 1	2 ± 1	3 ± 1
F. pratensis								
Preval [2]	13.8 ± 0.8	–	92 ± 2	–	–	–	–	–
Preval [1]	14.2 ± 1.2	–	82 ± 1	–	–	–	–	–

[1] $100\,kg\,N\,yr^{-1}$.
[2] $200\,kg\,N\,yr^{-1}$.

16:00 h. The daily palatability index [PI; equation (2)] was calculated according to the following formula[51]:

$$PI(t) = I_T(t)/I_C(t) \times 100 \qquad (2)$$

where $I_T(t)$ corresponds to the intake of the tanniferous forage eaten during the first t minutes divided by the total intake of tanniferous plants until 16:00 h. Analogously, $I_C(t)$ refers to the intake of the control forage eaten during the first t minutes divided by the total intake of the control forage until 16:00 h. The palatability index assumes that forage selection and intake rates at the beginning of a meal are good criteria to determine palatability of forage. The palatability index exceeds 100% when the percentage of the daily-consumed tanniferous forage already eaten after t minutes is larger than the corresponding percentage of the control forage. The palatability of the non-tanniferous control feed is defined as 100%, and tanniferous forages for which the palatability index exceeds 100% are more palatable than the control feed.

Antiparasitic activity of O. viciifolia *hay or silage*

The present feeding experiment aimed to assess the efficacy of dried or ensiled forage of *O. viciifolia* against gastrointestinal nematodes in sheep. Twenty-four Swiss white alpine × Swiss Black-Brown Mountain lambs (10 females and 14 males) were artificially infected with a single dose of *C. curticei* and *H. contortus* larvae. The lambs were between 2.5 and 3 months old and had a mean liveweight of 33 kg at the start of the experiment. Twenty-eight days post infection, the lambs were allocated to four comparable groups according to sex, bodyweight and fecal egg counts. For 16 days, the lambs were fed *ad libitum* with either *O. viciifolia* or a corresponding isoproteic and isoenergetic non-tanniferous control forage each as regular hay or silage, respectively. Fecal egg counts per gram dry feces were performed twice a week. Tannin concentrations of the forages were analyzed using a butanol–HCL assay with a *L. pedunculatus* standard[49].

Results and Discussion

Agronomic performance of tanniferous forage plants

O. viciifolia, *L. corniculatus* and *C. intybus* germinated and established well in 2004. In contrast, all cultivars of *L. pedunculatus* were soon outcompeted after an initially promising germination, mainly by unsown *Trifolium repens*. As a result, the DM proportions of *L. pedunculatus* were below 20% DM of the accumulated yield in 2005 (four harvests) for any of the cultivars even when they were sown as a monoculture (Table 1). Therefore, the following text will concentrate on results of *O. viciifolia*, *L. corniculatus* and *C. intybus* only.

The cultivars of *O. viciifolia*, *L. corniculatus* and *C. intybus* yielded between 9.9 and 13.0 t DM ha^{-1} yr^{-1} when sown in a monoculture (Table 1). Sowing the tanniferous plants in a mixture with *F. pratensis* increased the total yield markedly in the case of *O. viciifolia* (yields of mixtures ranged from 16.4 to 16.5 t DM ha^{-1} yr^{-1}), and even more so in *L. corniculatus* (yields of mixtures ranged from 17.3 to 18.4 t DM ha^{-1} yr^{-1}) but only slightly in *C. intybus* (yields of mixtures ranged from 12.5 and 12.8 t DM ha^{-1} yr^{-1}). In the case of *O. viciifolia* and *L. corniculatus*, yields of mixtures clearly exceeded the yields of both the respective monocultures of the tanniferous plant species as well as that of the grass monoculture. This transgressive overyielding resulted most likely from N_2-fixation[52] of the tanniferous legume species and from a spatial and temporal niche complementation with regard to growth.

For *O. viciifolia*, *L. corniculatus* and *C. intybus*, DM proportions of sown species in the yield ranged from 38 to 100% when the tanniferous cultivars were sown in a monoculture but between 89 and 100% when they were sown in a mixture with *F. pratensis* (Table 1). In other words, *F. pratensis* helped to reduce the invasion of weed and other unsown species both in terms of absolute and relative amounts of weed. However, the grass also reduced the proportion of sown tanniferous plant material in the harvest from 38–100% in monoculture to 6–94% in mixtures and, as a consequence, tannin concentrations in the harvest of mixtures were diluted accordingly (Table 1). Between species, differences in tannin concentrations at the yield level (Table 1) were much smaller than might have been expected from tannin concentrations of the tanniferous species alone (Fig. 1). While the yield of species with high tannin concentrations (i.e. *O. viciifolia* and *L. pedunculatus*) was at times heavily diluted by non-tanniferous plant material, this dilution was less severe in *L. corniculatus*, which has intermediate tannin concentrations and almost non-existent in the case of *C. intybus*, which has very low tannin concentrations.

Cultivar Visnovsky was clearly the most promising among the tested *O. viciifolia* cultivars. Visnovsky had the highest yield (when sown as a monoculture: 13.0; when sown as a mixture: 16.5 t ha^{-1} yr^{-1} DM) and the highest DM proportions of the sown plant species (monoculture: 76, mixture: 98% DM), the highest DM proportion of tanniferous plant material in the yield (monoculture: 76, mixture: 36% DM) and therefore also the highest tannin concentrations (monoculture: 24, mixture: 12 g kg^{-1} DM). For *L. corniculatus*, cultivar Lotar performed slightly better than cv. Oberhaunstädter and cv. Odenwälder. Lotar achieved a yield of 11.0 t ha^{-1} yr^{-1} in monoculture and 18.4 t ha^{-1} yr^{-1} in a mixture with *F. pratensis*. The proportion of tanniferous plants was 77% in monoculture and 32% when sown in a mixture and the tannin concentration of the yield (monoculture: 18, mixture: 10 g kg^{-1} DM) was only slightly lower than in *O. viciifolia*. Among the tested cultivars of *C. intybus*, the cultivar Puna clearly performed best. The other cultivars produced a large amount of stems, especially in the second year, adding to the DM yield and the DM yield proportions but supposedly reducing the forage quality.

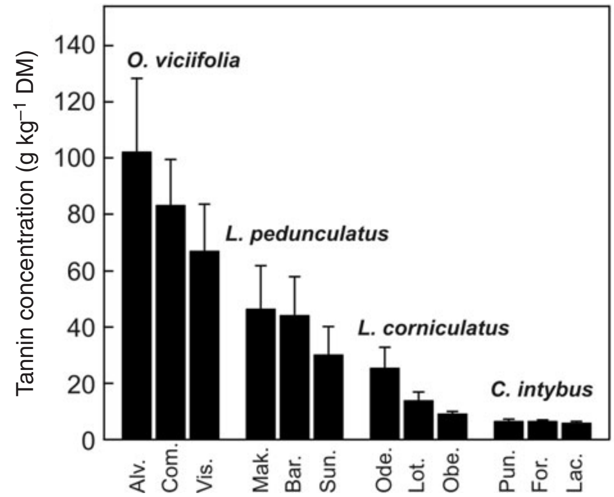

Figure 1. Tannin concentrations of harvestable aboveground biomass (>7 cm; mean ± SE; $n = 3$) of 12 field-grown tanniferous cultivars: *O. viciifolia*: ecotype Alvaschein (Alv.), commercial seed (Com.), cv. Visnovsky (Vis.). *L. pedunculatus*: cv. Grasslands Maku (Mak.), cv. Barsilvi (Bar.), cv. Grasslands Sunrise (Sun.). *L. corniculatus*: cv. Odenwälder (Ode.), cv. Lotar (Lot.), cv. Oberhaunstädter (Obe.). *C. intybus*: cv. Grasslands Puna (Pun.), cv. Forage Feast (For.), cv. INIA Lacerta (Lac.). All cultivars were collected on 8 May 2006.

Figure 2 displays the DM proportion of tanniferous plant material in each harvest and the corresponding tannin concentration for the most promising species and cultivars in this study (i.e. *O. viciifolia* cv. Visnovsky and *L. corniculatus* cv. Lotar). In both purely sown and mixed stands of *O. viciifolia* or *L. corniculatus*, respectively, fluctuations of DM proportions between tanniferous plants and non-tanniferous plant species were considerable. For example, in monocultures of *O. viciifolia* cv. Visnovsky, the proportion of tanniferous plants was 80% in August 2004, decreased to 55% DM in October of the same year but recovered to almost 100% DM in July of the following year. Shifts in the relative contribution of tanniferous plant material to total yield were reflected in the seasonal dynamics of tannin concentrations in the harvest. Therefore, we conclude that shifts in DM proportions and the competitive abilities of the tanniferous plant species are major drivers of seasonal fluctuations of tannin concentrations in the harvest.

Feed palatability

O. viciifolia had the highest concentration of condensed tannins, followed by *L. corniculatus*, while the tannin concentration of *C. intybus* was low. For all the investigated plant species, tannin concentrations (g kg^{-1} DM) were higher in silage (*O. viciifolia*: 100; *L. corniculatus*: 41;

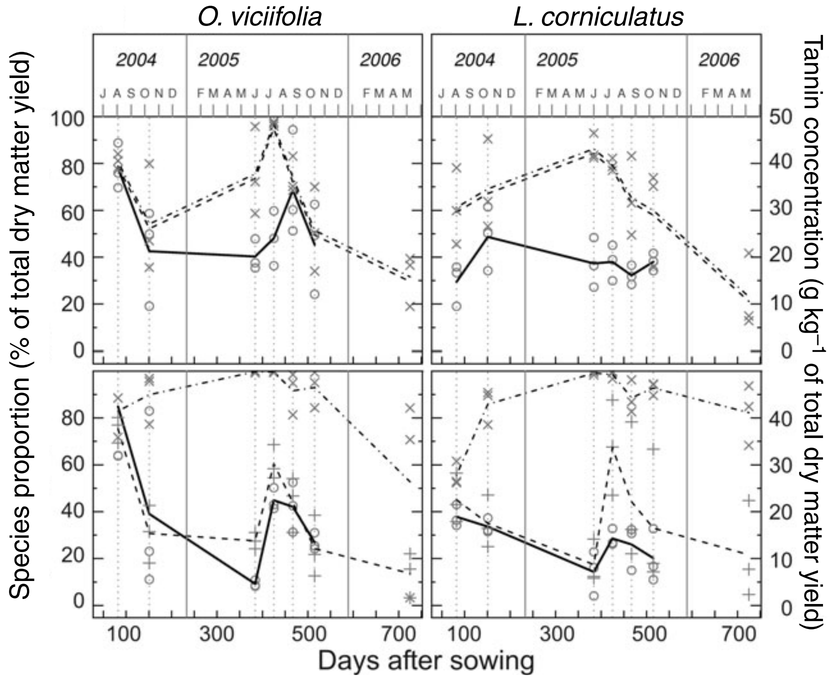

Figure 2. Mean proportion of sown species (dash-dotted line, $n = 3$), mean proportion of tanniferous species (dashed line, $n = 3$) as a percentage of the total DM yield, respectively, and the mean tannin concentration of individual yields (solid line, $n = 3$) in the course of the experiment. Upper row: purely sown stands of *O. viciifolia* cv. Visnovsky, *L. corniculatus* cv. Lotar. Lower row: the same species and cultivars as above but sown in a mixture with the grass *F. pratensis*. Original data on the plot level are presented as symbols (i.e. × = sown species, + = tanniferous species, ○ = tannin concentration).

C. intybus: 14) than in dried plant material (*O. viciifolia*: 92; *L. corniculatus*: 26; *C. intybus*: 11).

Offered as dried forages (Fig. 3a), palatability indices during the first 10 days were higher for *O. viciifolia* (PI: 91.2 ± 23.9%) and *C. intybus* (PI: 84.3 ± 23.0%) than for *L. corniculatus* (PI: 65.5 ± 21.8%). However, mainly due to very low initial values, none of the tanniferous forages was as palatable as the control forage when averaged over the first 10 days (the PI of the control is defined as 100%). By increasing the energy and nutrient supply 10 days after the start of the experiment, we expected the wethers to indicate the palatabilities of the different feeds more differentiatedly. However, during the second 10 days, the palatability indices of all the tanniferous forages approximated that of the control (i.e. 100%) to an even greater extent (average PI: *O. viciifolia*: 95.6 ± 2.9%; *C. intybus*: 102.9 ± 13.5%; *L. corniculatus*: 100.2 ± 13.1%). Hence, the palatability of the dried tanniferous forages was very similar to that of the dried ryegrass/clover mixture.

Fed as ensiled forages (Fig. 3b), the palatabilities of *O. viciifolia* (151.9 ± 81.9%) and *C. intybus* (121.2 ± 69.0%) were clearly higher than that of the control feed during the first 10 days of the experiment. Only *L. corniculatus* (PI: 77.7 ± 33.3%) had a palatability index lower than the ensiled control forage. During the second 10 days, *O. viciifolia* (on average: 159.6 ± 51.2%) remained more palatable than the control forage. Palatability of *C. intybus* (100.6 ± 16.2%) and *L. corniculatus* (101.0 ± 44.5%), however, were similar to that of the control forage.

The average palatability indices showed a high variation both among individual animals and between days. This variation was greater for ensiled than for dried forages, possibly due to the shorter evaluation time for the ensiled forage (7.5 min for ensiled forages versus 15 min for dried forages). In the case of dried tanniferous forages, palatability was initially low compared to controls. Already 2–3 days after the start of the experiment, however, the palatability of dried tanniferous forages did not differ from that of the familiar non-tanniferous control forage. Within this short period of time, adaptations in the wether's physiology or rumen flora, to avoid potentially detrimental effects of condensed tannins, are highly unlikely. It seems more plausible that the initially low but strongly increasing palatability indices indicate beneficial postingestive feedbacks or simply reflect a sensory customization to the unfamiliar diets. Wethers needed no time to become accustomed to ensiled tanniferous forages. They were at least as palatable as the control feed immediately after the start of the experiment.

It is concluded that after 2–3 days, and independent of the conservation method used, all tested tanniferous forage plants were at least similarly palatable as an equally conserved ryegrass/clover mixture. Ensiled *O. viciifolia* had the highest tannin concentrations of all the tested diets and regardless of its elevated tannin concentrations was clearly more palatable to the wethers than an ensiled ryegrass/clover mixture. Therefore, *O. viciifolia*, especially in its ensiled form, is considered the most promising

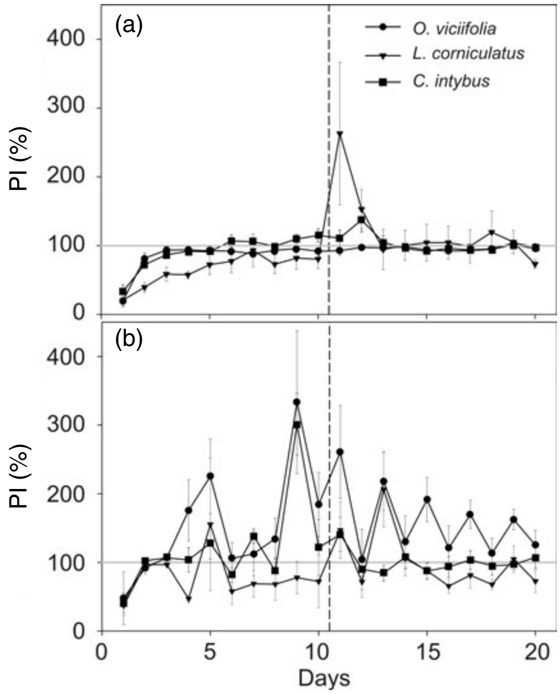

Figure 3. Palatability indices (PIs) of (a) dried or (b) ensiled tanniferous forage plants compared to a dried or ensiled non-tanniferous ryegrass/clover mixture (control), respectively. The palatability index of the control is defined as 100%. Tanniferous forages for which the palatability index exceeds 100% are more palatable to wethers than the control. From day 1 to 10, feeds covered 110% of the maintenance energy requirement. After day 10 (dashed line), energy supply was increased to 155% of the maintenance energy requirement (see the Methods section).

candidate for a practical application against gastrointestinal nematodes.

Antiparasitic activity of O. viciifolia *hay or silage*

The tannin concentrations of *O. viciifolia* hay and silage were 62 g kg^{-1} DM and 41 g kg^{-1} DM, respectively. Compared to their respective control groups, marked reductions in the combined fecal egg counts of *H. contortus* and *C. curticei* were observed when *O. viciifolia* was fed as hay or silage (Fig. 4). Within the 16-day feeding period, fecal egg counts decreased by 58% when lambs were fed with *O. viciifolia* hay, whereas fecal egg counts increased by 43% when lambs were fed with the control hay. For *O. viciifolia* silage, faecal egg counts were reduced by 37%, whereas in the corresponding control group fecal egg counts increased by 16%.

In comparison to the results of freshly administered *O. viciifolia*[53], the antiparasitic properties of *O. viciifolia* were largely preserved in both silage and hay, leading to a substantial decrease in worm egg excretion in feces. Our results suggest that the ensuing pasture contamination with infective larvae will decrease considerably and re-infections will be reduced when sheep are fed with *O. viciifolia*.

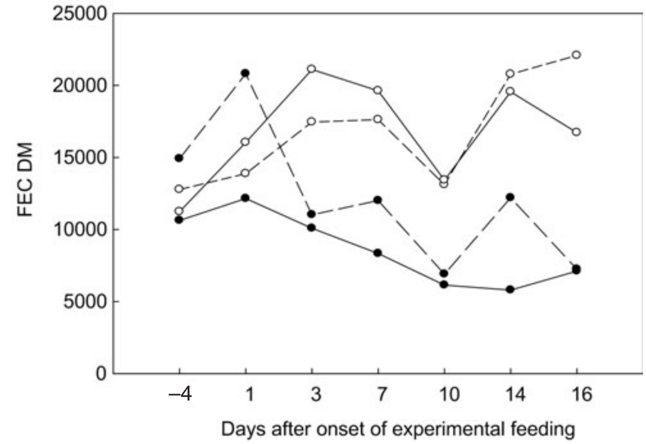

Figure 4. Mean parasite egg counts per gram dried feces (FEC DM; $n = 6$) of lambs either fed with *O. viciifolia* (closed symbols) or non-tanniferous control forage (open symbols), each administered as hay (dashed line) or silage (solid line), respectively.

Synthesis

O. viciifolia (cv. Visnovsky), *L. corniculatus* (cv. Lotar) and *C. intybus* (cv. Grasslands Puna) appeared particularly suitable for cultivation under the given temperate climatic conditions, whereas *L. pedunculatus* was competitively weak, possibly because this species prefers more humid than ‘average’ agronomic conditions. Similar performances of these species have been observed in an independent cultivation trial at Frick, Switzerland[53]. With regard to their tannin concentrations, only *O. viciifolia* and *L. corniculatus* seemed promising candidates for the control of gastrointestinal nematodes in ruminants, whereas tannin concentrations of *C. intybus* were very low. Nevertheless, *C. intybus* has repeatedly been reported to have anthelmintic and other desirable properties possibly related to elevated concentrations of other secondary metabolites (e.g. sesquiterpene lactones) found in this species[3].

Our field experiment demonstrated that the competitive ability of even the most promising tanniferous candidate plants (i.e. *O. viciifolia* cv. Visnovsky and *L. corniculatus* cv. Lotar) was suboptimal. As a consequence, seasonal fluctuations of tannin concentrations can, to a large extent, be attributed to shifts in the relative contribution of tanniferous plant material to total yield, even in purely sown swards. Pronounced short-term fluctuations in tannin concentrations of harvestable biomass (e.g. *O. viciifolia*, +79% within 17 days)[53] can result from differences in momentary growth rates between sown tanniferous plant species and their (sown or unsown) non-tanniferous neighbors. Additionally, fluctuations of tannin concentrations in pure stands can result from shifts in leaf/stem ratios of tanniferous forage plants during their development[14]. This implies that with regard to the suggested breeding of tannin-rich plants[1], a high competitive ability and a large leaf to stem ratio[14] are parameters likely to be just as important in enhancing the tannin concentration of harvestable biomass as an elevated intrinsic tannin concentration of the leaves of a cultivar.

With regard to the cultivation and management of tanniferous forage plants, mixtures were found to have clear advantages in relation to the total DM yield and in suppression of unsown species. Future research should focus on how to profit from the advantages of mixtures without suppressing tanniferous plants and lowering tannin concentrations. Possible options are (i) to lower nitrogen input to increase the competitive advantage of the tanniferous legume relative to the grass competitor, (ii) to reduce sowing densities of the grass, or (iii) to test mixtures with other grasses (e.g. short-bladed and slow-establishing grasses such as *Agrostis alba* or *Festuca rubra*). Alternatively, one could try to enhance the performance and reduce weed invasion in monocultures of tanniferous forages, for example (iv) by increasing sowing densities or (v) by adapting cutting frequencies to the tanniferous species.

Parallel to optimizing the cultivation and concentration of condensed tannins in the target candidates, investigations on the acceptance of these plants by sheep are of major importance. The feeding and palatability experiment showed that 2–3 days after the start of the experiment, the palatability of tanniferous forages was at least similar to a ryegrass/clover control, independent of the conservation method used. Regardless of its comparatively high tannin concentration, ensiled *O. viciifolia* was the most palatable among the tested forages. There was no evidence of negative side effects on the wethers from any of the tanniferous forages. Across species, the palatability of the different forages appeared unrelated to their tannin concentrations. If condensed tannins as 'plant defensive compounds'[15,17,18] should deter herbivores, they do not seem to have acted through a depressing effect on palatability or short-term physiological feedbacks in our experiment. It is also possible that the apparent lack of a 'plant defensive effect' of condensed tannin in this study was caused by interactions with other nutrients[11,54] or different chemical structures and 'potency' of tannins in the tested plant species[5]. In this context, the multitude of potentially influential chemical features of tannins and the great variety of suggested methods to analyze them contrasts with the fact that we still lack a simple but useful principle to interlink the chemical structure and the biological activity of tannins[5,11,55].

Apart from the general interest in the potential advantages and disadvantages of tanniferous forages for ruminant metabolism, the present study was primarily aimed at preparing a pathway towards their use against gastrointestinal nematodes. Administering of hay or silage of *O. viciifolia* to lambs co-infected with *H. contortus* and *C. curticei* reduced the combined fecal egg output markedly compared to the controls. As trichostrongylidosis (i.e. the disease caused by gastrointestinal nematodes) is a pasture-borne disease, a reduced contamination of the pasture with parasite eggs and infectious L3 larvae is likely to diminish the risk of renewed infections. Besides the effect on fecal egg output, there is strong evidence for a direct lethal effect of *O. viciifolia* on *H. contortus*[47,53], the species with the highest pathogenicity among the gastrointestinal nematodes of temperate and (sub)tropical regions[47].

Conclusions

The satisfactory yield and competitive ability of *O. viciifolia*, its high palatability despite its elevated tannin concentration and its efficacy against gastrointestinal parasites even in a conserved form would appear to make this tanniferous plant species an ideal candidate for the implementation of a non-pharmaceutical control strategy against gastrointestinal parasites. With regard to forage conservation, ensiling seems preferable to drying because it minimizes the loss of tannin-rich leaves, allows forage conservation relatively independent of the weather and, according to our results, does not diminish the palatability to sheep or the efficacy against intestinal parasites.

Further research is needed to improve our understanding of the relationship between the chemical features of the condensed tannins and their biological activity. In particular, it is essential to clarify the mode of action by which condensed tannins exert antiparasitic effects in sheep. Future studies should aim to enhance efficacy against gastrointestinal nematodes by focusing on the possibilities of increasing concentrations of condensed tannins in the offered forage without reducing the palatability or nutritional quality of the feed. Finally, a control strategy against gastrointestinal nematodes based on condensed tannins needs to be adapted to optimize its technical and economical practicability for employment and acceptance on private sheep farms.

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