

## Use of Tanniferous Plants against Gastro-Intestinal Nematodes in Ruminants

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### Abstract

On organic farms, infections of gastro-intestinal nematodes (GIN) in grazing cattle, sheep and goats can have a detrimental impact on animal health, and are therefore of substantial economic importance. The aim of our interdisciplinary project is to investigate the basic conditions for the implementation of a control strategy against GIN based on the use of tanniferous plants. The project combines research on plant growth and management, animal nutrition and parasitology. The first results, especially those from *Onobrychis viciifolia* (sainfoin), presented here are encouraging. *O. viciifolia* has a consistently high concentration of condensed tannins (CT) throughout the whole growing season. Thus, the optimal time for harvest can be determined in relation to agronomic properties such as fodder quality and yield and does not have to be related to a specific time period with high tannin concentration in the herbage. Despite its high CT content, the animals accepted *O. viciifolia* very well. Finally, the anti-parasitic properties of fresh CT-containing *O. viciifolia* were largely preserved in silage and hay leading to a substantial decrease in worm egg excretion in faeces. The present findings are promising for a potential integration of the CT-approach into the complementary control strategies against GIN in sheep.

### Introduction

On organic farms, infections of GIN in grazing cattle, sheep and goats can have a negative impact on animal health, and so they are of substantial economic importance. Organic farmers are confronted with the problem of (i) reducing the infection pressure to an acceptable level and (ii) having largely to resign themselves to the use of conventional anthelmintic drugs in keeping with the organic farming guidelines. As complementary control strategies such as homeopathy or phytotherapy have not yet achieved satisfactory control of internal parasites, the control of endoparasites remains largely based on the use of anthelmintics. The present situation is incompatible with the philosophy of organic animal husbandry and necessitates the development of alternative forms of parasite control.

Recent studies on plants containing condensed tannins (CT) have shown anthelmintic effects, which have been attributed to their elevated CT content (Kahn & Diaz-Hernandez, 2000; Min & Hart, 2003). Effects have been found against adult parasites *in vivo* as well as against larval stages *in vitro*. The use of tanniferous plants as forage crops can thus potentially be incorporated into a combined control strategy against GIN.

Before we can recommend the implementation of tanniferous plants in an integrated control strategy against GIN, the basic mechanisms have to be understood and the system has to be adapted to local conditions. In a four-year interdisciplinary research project, the efficacy and practicability of a CT-based control strategy against GIN are tested under temperate climatic conditions in Switzerland. The single modules address the topics of plant growth and management, animal nutrition and parasitology.

### Methodology

#### *Plant growth and management*

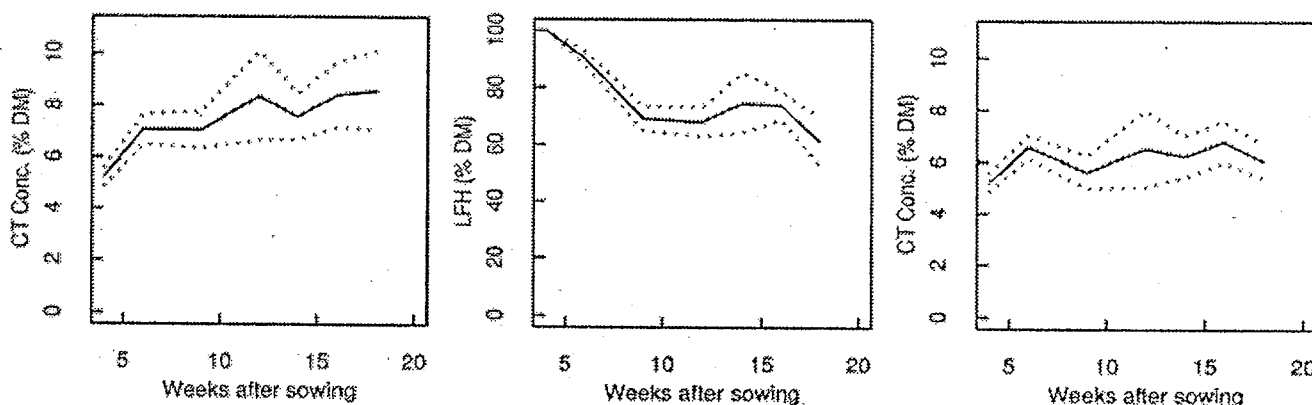
#### Aim and methods

Condensed tannins (CT) can have either beneficial or adverse effects on ruminants, depending on the concentration in which they are fed. Previous agronomic studies suggest that the optimal CT concentration is close to but below 5% DM (e.g. Aerts et al., 1999). Thus, we aimed to determine the optimal time for harvest with respect to tannin concentrations in three tanniferous plant species of the temperate regions for which positive effects on ruminants have been reported previously.

In an outdoor experiment *Onobrychis viciifolia* (sainfoin), *Lotus corniculatus* (birdsfoot trefoil) and *Cichorium intybus* (chicory) were sown in pots with a volume of 12 litres. The biomass allocation to leaves and stems, and the tannin concentration (Terrill et al., 1992) of the plants, were studied during the course of the vegetation period 2003 (from sowing until leaf senescence) in Zurich, Switzerland.

### Results and discussion

Over the entire vegetation period, concentrations of CT in the species for any given tissue were in the following order: *O. viciifolia* > *L. corniculatus* > *C. intybus*. The tannin concentration in leaves was roughly three times higher than in stems in *O. viciifolia* and *L. corniculatus* and similarly low in both tissues in *C. intybus*. In all the species investigated, the tannin concentrations of stems were stable over time. In contrast, the tannin concentrations of leaves increased in *O. viciifolia* (from 5 to over 8 % DM; Fig. 1) and *L. corniculatus* (from 2 to 5 % DM) during the experiment. However, as plants grew older the proportion of leaves – where most tannins are located – decreased in harvestable biomass from 100, 80, 100% to 60, 35, 80% for *O. viciifolia* (Fig. 1), *L. corniculatus* and *C. intybus* respectively. With regard to the overall tannin concentration of harvestable biomass, the increasing concentration of CT in the leaves was almost exactly evened out by a dilution effect due to the increasing proportion of ‘tannin poor’ stems in the harvest. As a result of these two effects, the tannin concentration of the harvest was nearly constant during the growing season for all investigated species at about 6 % DM in *O. viciifolia* (Fig. 1), 2.5 % DM in *L. corniculatus* and <1 % DM in *C. intybus*.



**Figure 1:** The concentrations of condensed tannins in leaves (CT Conc., left), the proportion of leaf fraction in harvestable biomass (LFH, middle) and the concentration of condensed tannins in harvestable biomass (CT Conc., right) during the experiment. All data from *Onobrychis viciifolia*. Shown are means and 95% confidence intervals of the mean.

In conclusion, as the CT concentration of harvestable biomass was found to be constant during the season, an optimal time for harvest can be determined in relation to agronomic properties such as fodder quality and yield. Of the investigated species, the tannin concentration in *O. viciifolia* seems most promising for the application against GIN.

### Animal Nutrition

#### Aim and methods

High dietary concentrations of CT were related to low voluntary feed intake (Titus et al., 2000). Nevertheless, we aimed to find tanniferous plants that are well accepted by the animal, by assessing the palatability of three tanniferous plant species, each fed as dried or ensiled forage, and to compare it with a non-tannin containing grass/clover mixture.

The preference for each of the three CT-containing forage plants (*O. viciifolia*, *L. corniculatus*, *C. intybus*) was tested against a control mixture of ryegrass, white clover and red clover with three groups of adult wethers (n = 6). The study comprised two consecutive experiments: in the first experiment the forages were fed dried, and in the second experiment as silage. The respective CT-containing forage and the control forage were offered simultaneously in two separate boxes. In the first 10 days of each experiment, the diets contained 110 % of the maintenance energy requirement (ME (MJ) = 0.38 x LW<sup>0.75</sup> x 1.1) and were given in equal portions twice a day. During the second 10 days, the sheep received half of the experimental diets in

the morning and low-quality hay in the evening. The total diets covered 155% of the maintenance energy requirement. The palatability index (PI; Salem et al., 1994) was calculated 7.5 min (ensiled forage) and 15 min (dried forage) respectively after morning feeding. The CT content of the forages was analysed according to Terrill et al. (1992).

### Results and discussion

On average, *O. viciifolia* (10 % DM) had the highest content of CT followed by *L. corniculatus* (3.3 % DM) and *C. intybus* (0.9 % DM). Averaged over all species, the CT content of dried forages (4.2 % DM) was slightly lower than that of silages (5.5 % DM).

Offered as dried forage, the preference of wethers for *O. viciifolia* (PI:  $91.2 \pm 23.9$  %) and *C. intybus* (PI:  $84.3 \pm 23.0$  %) was higher than for *L. corniculatus* (PI:  $65.5 \pm 21.8$  %) during the first 10 days (Fig. 2), but none of the CT-containing forages achieved the PI of the control forage (100 %). During the second 10 days, the PIs of the CT-plants (*O. viciifolia*:  $95.6 \pm 2.9$  %; *C. intybus*:  $102.9 \pm 13.5$  %; *L. corniculatus*:  $100.2 \pm 13.1$  %) were in the same range as the control forage (100%).

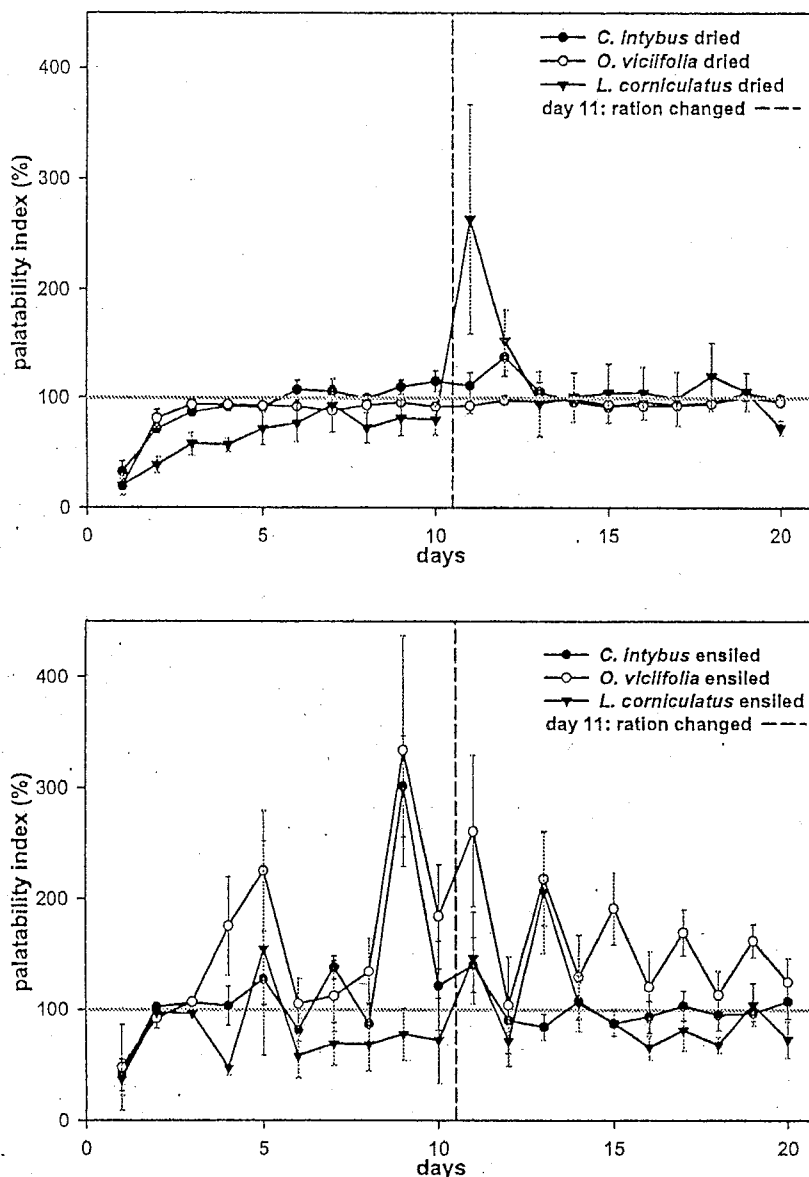


Figure 2: Palatability index (PI) of CT-containing plants compared to control forage (PI: 100 %) for dried (top) and ensiled (bottom) forages. Bars indicate standard errors.

Fed as ensiled forage, the PIs of *O. viciifolia* ( $151.9 \pm 81.9$  %) and of *C. intybus* ( $121.2 \pm 69.0$  %) were clearly higher than the PI of the control forage during the first 10 days (Fig. 2). In contrast, the preference for *L. corniculatus* ( $77.7 \pm 33.3$  %) was lower compared with the control forage. During the second 10 days, wethers clearly preferred *O. viciifolia* ( $159.6 \pm 51.2$  %) compared to the control forage while *C. intybus*

( $100.6 \pm 16.2$  %) and *L. corniculatus* ( $101.0 \pm 44.5$  %) were in the same range as the control forage. Regarding the PI of *L. corniculatus*, wethers seemed to need a longer time to become accustomed to this feed.

In conclusion, the preferences of the wethers for the plants were not related to their content of CT. Despite its high content of CT, *O. viciifolia* was very well accepted by the animals and when ensiled, it was even preferred to the control forage.

### Parasitology

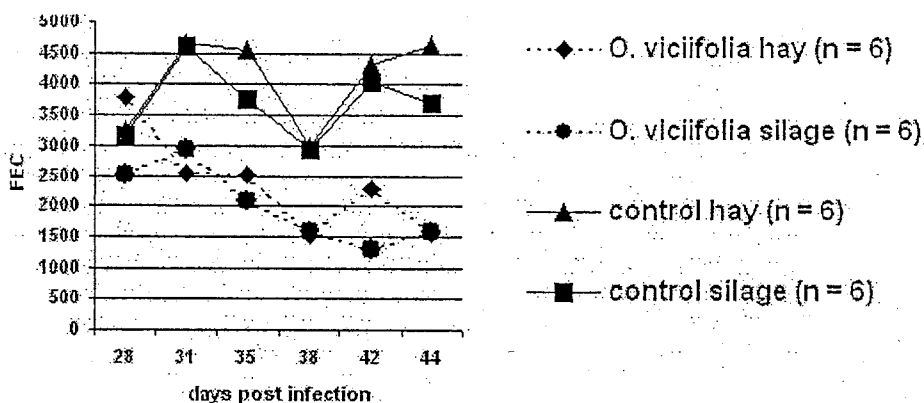
#### Aim and methods

A reduction of GIN egg counts in faeces (FEC) was observed in several *in vivo* trials with CT using sheep and goats (Min & Hart, 2003). Recent studies focused onto whether these effects also hold true for conserved CT-containing plant material. Single and repeated administration of *O. viciifolia* hay to goats, naturally infected with GIN, has proven effective in reducing FEC (Paolini *et. al.* 2005). However, no information is available on whether this is also true for (i) ensiled CT-containing plants and (ii) other ruminant hosts like sheep. Thus, the present feeding trial aimed to assess the efficacy of ensiled forage of *O. viciifolia* against GIN in sheep.

Twenty-four lambs of the white alpine breed were artificially infected with a single dose of *Cooperia curticei* and *Haemonchus contortus* larvae. Twenty-eight days post infection lambs were allocated to 4 equal groups according to bodyweight and FEC. For 15 days the lambs were fed *ad libitum* with either *O. viciifolia* or corresponding isoproteic and isoenergetic control fodder without CT each as regular hay or silage respectively. FEC were performed twice a week. CT-concentrations of the fodder were measured according to Terrill *et al.* (1992).

#### Results and discussion

For *O. viciifolia* hay and silage the CT concentrations were 6.2 % DM and 4.1 % DM respectively. Compared to the control groups a marked reduction in FEC was observed in both *O. viciifolia* groups (Fig. 3). Within the 15-day feeding period, FEC decreased by 58 % when lambs were fed with *O. viciifolia* hay, whereas FEC increased by 43 % when fed with the control hay (geometric means). For *O. viciifolia* silage, FEC was reduced by 37% whereas in the corresponding control group FEC increased by 16%.



**Figure 3: Geometric means of parasite egg counts in faeces (FEC) of lambs from 4 groups. Lambs were experimentally infected with *H. contortus* and *C. curticei* at day 0. Hay or silage was fed from day 29 to day 43 post infection.**

In conclusion, the anti-parasitic properties of fresh CT-containing *O. viciifolia*, which were documented previously, are largely preserved in both silage and hay; leading to a substantial decrease in worm egg excretion in faeces. This implies that the ensuing pasture contamination with infective larvae will decrease considerably and reinfections will be reduced.

## Conclusion

The present findings offer exciting opportunities for the practicability of the approach of feeding *O. viciifolia* against gastro-intestinal nematodes. Silage and hay are easily produced and the administration to sheep is neither linked to grazing nor to the vegetation period. Furthermore, as bioactivity is maintained after storage and transport of the conserved material, the production of *O. viciifolia* can be centralized.

## Acknowledgements

We thank F. Wernli for checking the English. The project is supported by the Swiss Federal Office for Agriculture.

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