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# Evapotranspiration affects condensed tannin levels and MPP differently in four grassland mixes

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## Abstract

Dairy farming faces challenges of summer fodder scarcity and methane emissions. To address these issues, one may incorporate plants rich in condensed tannins (CT) into temporary grassland mixes. However, knowledge regarding the link between CT, milk production and climate remains limited in a field context. We conducted an experiment with four grassland mixes (grass, grass and legumes, grass and plants rich in essential oils, grass and plants rich in tannins) replicated four times over six rotations. CT content, dry matter yields, and botanical composition were analysed. We calculated milk production potential (MPP) based on nutrient analysis of the plant material and obtained potential evapotranspiration (PET) data. Results showed no CT difference across mixes in low PET rotations. However, during a high PET rotation CT contents in the tannin-rich mix were higher than the others. MPP remained stable over time for the legume mix, the mix with plants rich in essential oils, and tannins but decreased significantly for pure grass. CT content in the tannin mix was strongly correlated with *Lotus corniculatus* abundance. Our study suggests that plants produce the most CT during peak PET in summer, but forage production is highest during lower PET in spring. Thus, tannin-rich mixes may primarily mitigate methane emissions in summer. Importantly, MPP from tannin mixes did not decrease with increasing PET, highlighting the potential protective role of condensed tannins on plants during summer drought.

**Keywords:** multi-species grassland, condensed tannins, evapotranspiration, lotus corniculatus

## Introduction

Pastures characterized by a higher diversity of plant species have been proposed to facilitate increased nutrient absorption by ruminants and promote better cattle health (Distel et al., 2020). This effect is due in part to the absorption of plant secondary metabolites such as condensed tannins (CT). These tannins, or proanthocyanidines, are polyphenolic compounds found in various plant species, in which they act as defence mechanisms against biotic (Barbehenn & Peter Constabel, 2011) and abiotic stress such as drought (Gourlay et al., 2022). CT could potentially reduce methane emissions from cattle during digestion by inhibiting certain microorganisms involved in methane production in the rumen (Wang et al., 2015). On-field studies on this topic remain scarce, highlighting the importance of comparing forage production and quality in grassland mixes with tannin-rich plants versus conventional blends. We anticipated that a grassland mix enriched with tannin-rich plants would consistently display higher CT levels throughout the vegetation season, given the intentional selection of tannin-rich plant species. Concurrently, we expected these CT levels to correlate with PET. In addition, traditional blends, selected for their productivity, were predicted to sustain elevated productivity across the entire vegetation period.

## Materials and methods

The study was conducted during a single growing season from the end of March to October 2022 in Frick, Switzerland (47°30'51"N 8°01'26"E). The experimental field, covering

approximately 1.3 hectares, was divided into 16 plots. In the autumn of 2021, four grassland mixes were randomly allocated and sown on four plots each: grass, grass and legumes, grass and plants rich in essential oils, grass and tannin-rich plants (in the following referred to as Grass; Legume; Tannin and Oil mixes). Throughout six grazing rotations, we closely monitored the botanical composition, the CT content (in  $\text{g}\cdot\text{kg}^{-1}$ ) and the dry matter yield (in  $\text{kg}\cdot\text{ha}^{-1}$ ) of each plot. Furthermore, we calculated the milk production potential (MPP) of a hypothetical dairy cow (in  $\text{kg}\cdot\text{ha}^{-1}$ ) using the INRA (2010) equation. PET was assessed (agrometeo.ch). All statistical analyses were conducted using R. Given the non-normal distribution of our data, we employed Spearman tests to assess the correlation between CT levels and potential evapotranspiration (PET), between MPP and PET (for each mix) and between the relative abundance of supposedly tannin rich species (only in Tannin mix) and CT levels. Wilcoxon pairwise comparison tests were executed to discern variations in CT and MPP between the mixes throughout the six rotations.

## Results and discussion

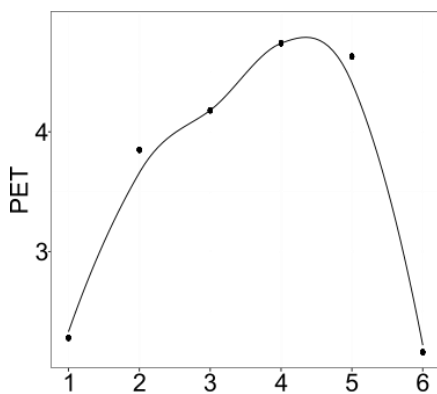


Figure 1-Variation of the PET along the 6 rotations

The PET exhibited an increase during the first four rotations, spanning from the end of March to the end of June. Rotations 4 and 5 were conducted during the period when PET reached its peak. Subsequently, there was a decline observed from rotation 5 (August) to rotation 6 (October) (Fig. 1). There were significant positive correlations between CT and PET in each of the four mixes (Table 1). The strongest correlation was observed in the Tannin mix.

Table 1- Results of the Spearman correlation tests between CT and PET in each of the mixes

Mix	Spearman correlation coefficient ( $\rho$ )	S	P-value
Grass	0.46	1237.8	0.023*
Legume	0.55	1043.5	0.006**
Tannin	0.84	362.56	<0.001***
Oil	0.41	1359.7	0.047*

Significant differences in CT levels among the four mixes were observed only at Rotation 5, where the Grass mix and the Legume mix exhibited significantly higher CT levels than the Grass mix, and the Tannin mix demonstrated significantly higher CT levels than each of the other three mixes (Figure 2a). During rotation 4, CT levels were highest in the Tannin mix but it did not differ significantly between the mixes. This lack of significance could potentially be attributed to soil heterogeneity and/or plant composition. In the Grass mix, there was a significant negative correlation between MPP and PET ( $\rho=-0,47$ ,  $S = 3370$ ,  $p\text{-value} = 0.02197$  \*). Significant differences in MPP among the four mixes were observed only at rotation 1, where the Grass and the Legume mixes exhibited significantly higher MPP than the Tannin and the Oil mixes (Fig. 2b). These findings suggest that the initial highest yields of the Grass mix were later negatively impacted by the PET. It also appeared that the MPP of the Grass mix were

lower in the rotation 5 than for the other mixes but the correlation was not more than marginally significant.

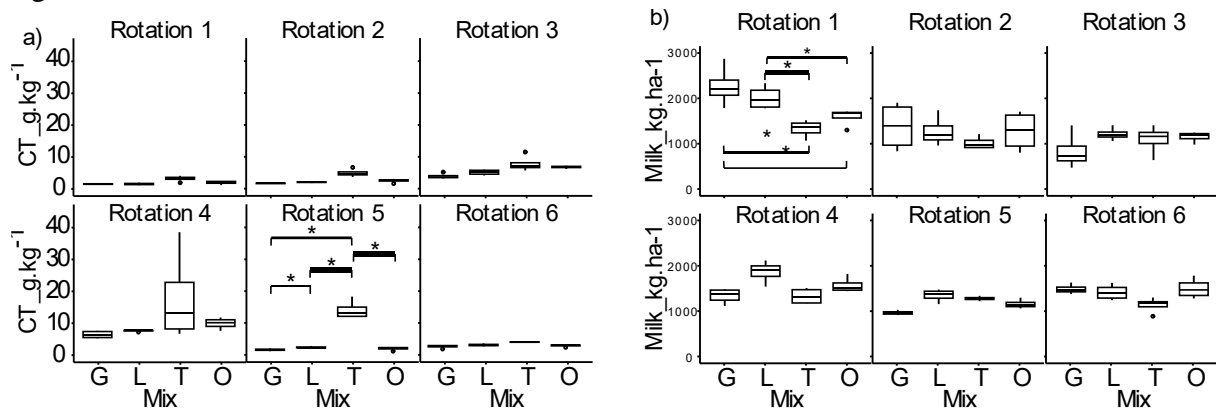


Figure 2- CT levels in  $g.kg^{-1}$  (a) and Milk yields (b) in each of the mixes and rotations. Significant  $P$ -values from the Wilcoxon pairwise comparison tests are depicted by the corresponding “\*”.

Within the potentially tannin rich plants of our Tannin mix, only the relative abundance of *Lotus corniculatus* and the CT levels correlated significantly ( $\rho = 0.82$ ,  $S = 419.59$ ,  $p\text{-value} = 1.074e-06^{***}$ ).

## Conclusion

Conformingly to our hypothesis CT were correlated with PET in all mixes. However, in contradiction with what we expected, the Tannin mix was showing higher CT levels than the other mix only in rotation 5. This result could be explained by the higher *Lotus corniculatus* abundance in rotation 5 as this species has the ability to sustain higher PET. This implies that using tannin-rich species to modulate digestive processes in the rumen, i.e. reduce methanogenesis or improve protein digestibility, may only be effective during periods with high PET. Surprisingly, the Grass and Legume mixes displayed higher MPP than the other mixes only at Rotation 1. This could be partially explained by the adverse impact of PET on Grass, along with the Tannin and Oil mixes' ability to maintain yields despite higher PET. In conclusion, our results underscore the significance of climate and timing in designing grassland systems that offer multiple services.

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