**Association between the** **sowing site and methanogenic potential of seven forage species**

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**Application**

Sowing site could modulate enteric methane production by changes in the chemical composition of forages. Therefore, sowing site is a factor to consider when evaluating the methanogenic potential of forages.

**Introduction**

As a greenhouse gas, enteric methane (CH4) emissions have a significant environmental impact and represent an energy loss for ruminants. Nevertheless, feeding high quality forages are related to lower CH4 emissions (Vargas et al., 2022), which are high in crude protein (CP), and low in neutral detergent fiber (NDF). Forage species under the same management, but growing in different locations, exhibit a different chemical composition due to differing environmental conditions of each site, and, therefore, it is of interest to evaluate their CH4 production. The objective of this experiment was to evaluate the *in vitro* CH4 production of seven forage species used in ruminant production, growing in two locations contrasting in temperature and rainfall.

**Material and methods**

The methanogenic potential of seven forage species was evaluated using a discontinuous fermentation system (batch). The forage species evaluated were Alfalfa (AF; *Medicago sativa*), Bromegrass (BG; *Bromus* spp.), Festulolium (FL; X *Festulolium*), Orchardgrass (OG; *Dactylis glomerata*), Ryegrass (RG; *Lolium perenne*), Tall fescue (TF; *Festuca arundinacea*), and the root bulb and leaves of Turnip (TUR; *Brassica rapa*). Forages species were sown in 2019 at Icalma (-38°49'11.1" S; -71°17'26.6" W; 1160 m above sea level (ASL)) and Pehuenco (-38°36'44.4" S; -71°7'18.8" W; 900 m ASL), both sites located in the Andes foothills, La Araucanía, Chile. A randomized block designs with three blocks was used at each site, and within each block the forage species were randomly distributed. All grass species were harvested in January 2021, while AF and TUR were harvested in March 2021. Samples were dried in oven and ground, and field replicates from each site were pooled by species and incubated separately in bottles (n=32). The inoculum was a mixture of rumen fluid from two ruminally cannulated Holstein cows and medium of Mould. In a 1:2 volume ratio. The inoculum was transferred to incubation bottles under CO2 and incubated at 39 °C for 24 h. Gas production (GP) was measured with a pressure transducer and a sample was extracted into previously evacuated exetainers for subsequent analysis of CH4 and hydrogen (H2) concentration by gas chromatography. Bottles were then opened and pH immediately measured. Bottle contents were centrifuged, the supernatants discarded, and the pellets dried to determine the dry matter (DM) disappearance (DMD). In addition, each forage species was analyzed for its content of NDF, CP and ash. Organic matter (OM) was estimated as 100 – ash. The relationship between variables was studied through Pearson correlations (r), and visualized using Principal Component Analysis (PCA) Biplot. Significance was declared at *P* < 0.05, and trend at 0.05 ≥ *P* < 1. Data were analyzed with JMP software ver. 16.1.

**Results**

The *in vitro* CH4 production varied among sowing sites and forage species. In general, the species from Icalma produced in average more CH4 that the same species from Pehuenco (35.6%). In Icalma, AF and grass species (BG, FL, OG, RG and TF) produced 41.1% and 3.1% CH4,respectively. While in Pehuenco, those species produced 67.5 and 12.3% CH4, respectively. At both sowing sites, AF produced more CH4 (52.3%), grass species less CH4 (-0.3%) and TUR lesser CH4 (-25.5%) than the overall mean. In case of grass species, RG and TF produced more CH4 than FL and OG in both sites. The PCA Biplot (Figure 1) illustrate that 73.2% of the variation was explained by the first two principal components (PC1 and PC2, respectively). The score plot of PC1 discriminated TUR (leaves and root bulb) from other forage species and sowing site. In contrast, score plot of PC2 only discriminated based on sowing site. Independent of components, Icalma had an opposite direction to Pehuenco. The magnitude of vectors are similar among variables with exception of DM, but not directions. The Vectors showed that CH4 production trend to be positively associated with OM (r = 0.45; *P* = 0.078). In opposite, CH4 was negatively associated with H2 (r = –0.83; *P* < 0.0001) and CP (r = –0.34; *P*=0.193). Gas, NDF and pH exhibited lower association with CH4.

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**Figure 1.** Principal Component Analysis Biplot of chemical composition of forage species and ruminal fermentation variables.

**Conclusions**

The *in vitro* CH4 production between Icalma and Pehuenco sowing site were dissimilar. There also are difference *in vitro* CH4 production in forages species, where AF, TF and RG produced more CH4 than other species and TUR produces less CH4 than other forages species. The *in vitro* CH4 are more associated with OM and directly opposite to H2 and CP.

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**Reference**

Vargas, J., Ungerfeld, E., Munoz, C., DiLorenzo, N., 2022. Animals 12, 1132.

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