

ELLAGIC ACID AND GALLIC ACID CAN MITIGATE METHANE PRODUCTION AND AMMONIA FORMATION IN AN IN VITRO MODEL OF SHORT-TERM RUMEN FERMENTATION

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Livestock production is of particular importance for human nutrition, but it has a high environmental impact. In particular, ruminants are the major contributors to the total livestock greenhouse gas emissions [1-2]. Several feeding strategies aimed to decrease the environmental footprint of ruminants were and are currently investigated. One of the most promising strategies is based on the dietary supplementation with tannins, that can mitigate methane production and ammonia formation, but also affect feed digestibility in ruminants [3]. The aim of this study was to assess the effect of dietary supplementation with two metabolites of hydrolysable tannins, ellagic acid (EA) and gallic acid (GA), on gas production using an in vitro rumen fermentation system. Rumen fluid was collected from one of four fistulated, lactating cows in each of the four runs. Fermenters were fed with a standard diet (200mg DM hay). Five different supplementation conditions were investigated (% of DM): i) EA 7.5, ii) EA 15, iii) GA 7.5, iv) GA 15, v) EA 7.5 + GA 7.5. After an incubation of 24 h at 39°C, several parameters were measured with the rumen samples: pH, ammonia, total SCFA production, microbial count (bacteria and protozoa) and in vitro dry matter digestibility (IVDMD). After gas phase collection, total gas production, methane and CO₂ were evaluated using gas chromatography. The treatments did not affect microbial count and pH (P>0.05). Total SCFA production decreased by 10% after all treatments (P<0.001). Total gas production decreased by 10% after all treatments (P<0.05). Methane production was significantly decreased by EA 15 (-20%, P< 0.001) and EA+GA (-25%, P< 0.001) treatments. CO₂ production was also decreased (P<0.001), but to a lesser extent as compared to methane. Ammonia production was significantly decreased by EA 15 (-13%, P<0.001) and EA+GA (-20%, P<0.001). All the treatments except GA 7.5 caused a slight but significant 10% reduction of IVDMD (P<0.001). These results showed that EA and GA can decrease gas production but affect digestibility of dry matter. The high doses of EA and GA were used to mimic a diet including natural extracts rich in hydrolysable tannins. Indeed, further work will concentrate on the dosage of tannin-secondary metabolites from rumen samples and on long-term in vitro incubation screening also with lower doses of metabolites, in order to see whether the decrease in gas production and decrease digestibility can be dissociated.

[1] Tapio et al. The ruminal microbiome associated with methane emissions from ruminant livestock, *J Anim Sci Biotechnol*, 8:7, 2017.

[2] Jayanegara et al. Divergence between purified hydrolysable and condensed tannin effects on methane

emission, rumen fermentation and microbial population in vitro. *Anim Feed Sci Technol*, 209:60–68, 2015.

[3] Aboayage et al. Potential of Molecular Weight and Structure of Tannins to Reduce Methane Emissions from Ruminants: A Review. *Animals*, 9:856, 2019.