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Effect of plant extracts on *in vitro* rumen fermentation and methanogenesisMusco N.¹, Cutrignelli M.I.¹, Boussaada A.², Calabrò S.¹¹Department of Veterinary Medicine and Animal Production, University Federico II, Napoli, Italy²Department of Veterinary Medicine, Batna 1 University, Batna, Algeria

Introduction: Recently, numerous studies have shown that plant secondary metabolites (i.e., saponins, tannins, phenolics) improve the efficiency of rumen fermentation, enhancing protein metabolism and decreasing methane (CH₄) production^{1,2}, one of the most important of greenhouse gases (GHG). However, it is not always easy to reduce environmental impact and, at the same time, preserve the nutritional value of the diet. Aim of the investigation was to compare the effects of two plant extracts on *in vitro* fermentation characteristics including CH₄ production. The hypothesis was that both extracts influence CH₄ production.

Animals, material and methods: The ethyl acetate extracts of leaves of *Eucalyptus globulus* (EG) or *Olea europaea* (OE) rich in flavonoids were used.³ About one g of standard diet (Forage: Concentrate ratio 60:40, CP 12.8 and NDF 43.9 % of DM) was incubated alone (Control) and with 50 mg of EG or OE extracts at 39°C, under anaerobic conditions with 10 ml of buffered rumen fluid, collected by four cows at slaughterhouse, according to the *in vitro* gas test method. After 24 hours the following parameters were determined⁴: total gas related to incubated organic matter (GP24, ml/g of OM) by a pressure transducer, degraded OM (OMD, %) by filtration, CH₄ (ml/g) and volatile fatty acids (VFA, mM/g) by gas-chromatography and ammonia (N-NH₃, mM/g) by colorimetric method. The differences between the two extracts were statistically tested (PROC GLM, SAS, 2000).

Results and discussion: Compared to control, both extracts did not influence (P>0.05) *in vitro* fermentation in terms of gas production (105, 109 and 110 ml/g for control, EG and OE extract, respectively). As reported by some authors⁵, an inclusion of plant extract might affect OM digestibility; in this study, only OE extract significantly (P<0.05) reduced OMD (58.8, 58.6 vs. 53.7 % for control, EG and OE extract, respectively). The same trend was observed for VFA production (P<0.01): 83.0, 81.6 vs. 70.0 mM/g for control, EG and OE extract, respectively. The N-NH₃ results are not easy to explain: its production was significantly (P<0.01) reduced by EG extract (32.5 mM/g) and increased by the adding of OE extract (75.7 mM/g) compared to the control (57.0 mM/g). Regarding CH₄ production, only EG extract consistently (P<0.001) decreased its production (1.14 ml/g) compared to control (14.6 ml/g) and OE extract (14.1 ml/g), as also reported by many authors.^{6,7} Ethyl acetate extraction allows the dissolution of mainly rich in hydroxyl radical compounds (eg. glycosylated flavonoids) that inhibit the *Archaea* fixation to protozoa.⁸

Conclusions: The addition of ethyl acetate extracts affects some *in vitro* fermentation characteristics, even if the results showed marked differences between the two plants. Therefore, different plant extracts might favour or depress bacterial activity. Further studies are necessary to identify the best extract that reduces GHG emission without lowering the rumen fermentation.

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