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Effects of sunflower oil infusions of *Asparagopsis taxiformis* on *in vitro* rumen methane production and biohydrogenation of polyunsaturated fatty acids

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Keywords: *Asparagopsis taxiformis*, biohydrogenation, sunflower oil, *in vitro*, methane

Asparagopsis taxiformis inhibits ruminal methane (CH₄) production due to its content in bromoform (CHBr₃) and its immersion in edible vegetable oils extracts and stabilises the highly volatile CHBr₃. The effects of this oil on *in vitro* rumen methanogenesis and biohydrogenation were studied. Five 48h *in vitro* batch incubations were performed using a total mixed ration diet as substrate without oil (Control) or with 60 µL of sunflower oil per g of substrate dry mass, differing in CHBr₃ content: 0 µg, 25 µg, 50 µg, 75 µg, 100 µg and 150 µg of CHBr₃. Organic matter degradability (OMD), total gas, CH₄, volatile fatty acids (VFA) and long-chain fatty acids (FA) were analysed. OMD was higher in Control than in oil-containing treatments and was unaffected by CHBr₃. Total gas was unaffected by treatments. Methane production had a quadratic decrease with increasing CHBr₃ concentrations, with relevant reductions only for dosages above 75 µg. Total VFA was unaffected by oil inclusion and CHBr₃. The molar percentage of acetic acid decreased linearly, while propionic, butyric and valeric acids increased linearly with CHBr₃. Increasing CHBr₃ dosage decreased linearly 18:0 and increased trans-18:1 FA, with 18:1t11 increasing linearly and 18:1t10 quadratically. The results provide evidence that oil immersions of *A. taxiformis* can successfully inhibit *in vitro* ruminal production of CH₄ without disturbing fermentation whilst modulating biohydrogenation.

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Effect of supplementation with oil enriched in bromoform from *Asparagopsis taxiformis* of lambs diet on productive performance and methane emissions

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A bromoform-enriched oil (Bromoil), prepared through immersion of *Asparagopsis taxiformis* in sunflower oil, was given to lambs to evaluate their growth performance and methane production. Twenty-four ram weaned lambs were housed individually in pens and fed ad libitum a low-starch total mixed basal diet supplemented with bromoil to attain 0, 15, 30 and 45 mg of bromoform per kg of feed dry matter. Diets were prepared daily using an experimental mixer by mixing 1.5 % of oil in the basal diet, adjusting the bromoform concentration in the oil to obtain the pretended levels of bromoform. The feed intake was controlled daily and live weight weekly. The lambs were slaughtered after 5 weeks and after slaughter the rumen mucosa was observed and sampled for histopathological analysis. Rumen contents of each lamb was collected immediately after slaughter and used as inoculum in a ANKOM *in vitro* system using the respective experimental diet as substrate in order to quantify the methane and total gas production after 24 hours of incubation. Growth performance was negatively affected by bromoform. Growth rate was higher in the 0 and 15 mg/kg than for 45 mg/kg treatments and was intermediate for the 30 mg/kg treatment. Intake was also higher for the 0 and 15 mg/kg than for 30 and 45 mg/kg treatments. Daily bromoform intake was 1.0, 19.0, 31.7, and 41.4 mg in treatments 0, 15, 30, and 45, respectively. Rumen mucosa observation revealed severe lesions located in the ventral region of all the lambs fed 30 and 45 mg/kg diets. In the lambs fed with 15 and 0 treatments the rumen wall presented a normal condition. Total gas production was not affected by treatments but methane production was 72 % lower for 30 and 45 mg/kg treatments when compared with 0 and 15 mg/kg treatments.

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