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Abstract of Thesis/Dissertation

Applicant

Doctoral Program in **Animal and Food Hygiene**

Graduate School of Animal Husbandry

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Title : The effect of natural feed additives on methane emissions, nutrient intake, digestibility and rumen fermentation parameters

(天然素材からなる飼料添加物の反芻家畜への給与が，消化管からのメタン産生，養分摂取，飼料消化率および第一胃内発酵に及ぼす影響)

Abstract

A series of studies were conducted to evaluate the effect of incorporating natural feed additives in the ration of sheep on methane (CH_4) emission, nutrient intake, digestibility, nutrient balance, volatile fatty acid (VFA) concentration, ammonia-N (NH_3-N) concentration, and the protozoa population. The study consisted of four experiments based on *in vitro* and *in vivo* techniques. The natural feed additives considered were Sunphenon 30S-O and Euglena. Sunphenon 30S-O is obtained from the leaves of green tea (*Camellia sinensis*) and standardized for its catechin content (210 g/kg dry matter (DM)). Euglena (*Euglena gracilis*) is a unicellular organism obtained in powder form with 100% purity from euglena Co., Ltd., Japan.

The first experiment was conducted to evaluate the effect of different concentrations of Sunphenon 30S-O using *in vitro* continuous gas quantification system and *in vitro* digestion techniques. The treatments considered were Sunphenon 30S-O at concentrations of 0.0, 20, 40, and 50 g/kg DM of Guinea grass (*Panicum maximum*) hay. Treatments with buffered rumen fluid were incubated for 24 h using *in vitro* continuous gas production and *in vitro* digestion techniques. The second experiment was conducted on *in vivo* evaluation of Sunphenon 30S-O in which four Corriedale weathers sheep were arranged in a 4x4 latin square design and fed a basal diet of Guinea grass (*Panicum maximum*) hay at the maintenance level with four different concentration of Sunphenon 30S-O (0, 10, 25 and 40 g/kg DM intake). The experiment was conducted for 84 days in four 21 day periods that consisted of 14 days of acclimatization, five days of measurement and two 24-h run in open-circuit respiration chambers to measure gas exchange. The data were subjected to polynomial regression analysis. My findings indicated that nutrients (dry matter (DM), organic matter (OM), crude protein (CP) and

gross energy (GE) intake declined linearly ($P<0.01$) and quadratically ($P<0.05$) with increasing concentration of Sunphenon 30S-O. On the other hand the apparent nutrient digestibility was not affected regardless of the concentration of the supplement. *In vivo* CH_4 emission (l/kg digestible OM intake) reduced linearly ($P<0.05$) by up to 13.5% with increasing concentration of the Sunphenon 30S-O and a similar trend was observed in *in vitro* CH_4 emission. Urinary and CH_4 energy loss decreased linearly ($P<0.01$) from 17.4% to 11.2% and from 7.3% to 6.2% of the GE intake, respectively, with increasing concentration of the supplement. The *in vitro* study indicated that VFA (mmol/L), NH_3-N concentrations (mg/ml) and protozoa population were reduced (linear $P<0.01$; quadratic $P<0.01$) with increasing concentration of Sunphenon 30S-O. The findings of this study showed that the presence of catechin, which is the precursor of condensed tannin, is responsible for reducing CH_4 emission from sheep.

The third experiment was conducted to investigate the effect of different concentrations of Euglena on CH_4 production, nutrient digestibility, total VFA and NH_3-N concentration as well as on the protozoa population. The treatments considered were Euglena at concentrations of 0.0, 50, 100, 200, 400 and 1000 g/kg DM of the substrate (60:40 forage: concentrate ratio) incubated for 24 and 96 h using an *in vitro* continuous gas production and *in vitro* two-stage digestion procedure, respectively. Methane emissions (ml/g DM) decreased at an increasing rate, generally with increasing concentration of Euglena but also exhibited quadratic ($P<0.001$) and cubic ($P<0.001$) effects while NH_3-N (mg/ml) concentration increased at an increasing rate (linear $P<0.001$; quadratic $P=0.001$; cubic $P=0.024$). Total VFA concentration (mmol/l) decreased significantly ($P<0.001$), when the substrate was totally replaced by Euglena. There was a linear ($P<0.001$) and cubic ($P=0.047$) reduction in protozoa population. *In vitro* DM digestibility was improved (linear $P=0.003$; quadratic $p=0.04$; cubic $P<0.001$). These findings suggest that Euglena at concentration of 100 g/kg DM reduce CH_4 emissions by 9.1% and improve DM digestibility by 15.3%. However, when the concentration of Euglena increases, while further reducing CH_4 emissions, have negative effect on NH_3-N concentration, protozoa population and VFA concentration. Euglena is rich source of amino acid and fatty acids, and the presence of higher proportion of saturated medium chain fatty acids in Euglena affected ruminal protozoa activity with subsequent impact on CH_4 emissions

In the fourth experiment, feeding trial was conducted to evaluate the effect of supplementation with different concentration of Euglena on nutrient intake, digestibility, nitrogen balance and NH_3-N concentrations. Four rumen cannulated Corriedale wethers sheep with an average body weight of 44.3±3.9 kg were arranged in a 4×4 Latin square design and fed a basal diet of Guinea grass (*Panicum maximum*) hay and concentrate mixture at the maintenance level with four different concentration of Euglena (0, 50, 100 and 150 g/kg DM intake). The experiment was conducted for 80 days in four 20 day periods that consisted of 14 days of acclimatization, 5 days of measurement and 1 more day for rumen liquor sample collection. The data were subjected to polynomial regression analysis. Dry matter, OM, acid detergent fibre (ADF) and GE intake increased linearly and quadratically ($P<0.05$) with increasing concentrations of Euglena. Similarly CP intake was increased linearly ($P<0.01$). Conversely DM, OM, NDF, ADF and GE digestibility were not influenced by supplementation of Euglena ($P>0.05$). Ruminal NH_3-N concentration increased (linear, $P<0.01$) while ruminal protozoa

population reduced linear and cubic ($P<0.01$) with increasing doses of Euglena. Apparent CP digestibility increased linearly ($P<0.01$). As a result, protein retention (g/d) increased linearly ($P<0.01$) and quadratically ($P<0.05$). Euglena supplementation at different concentration did not change ($P<0.05$) the total VFA concentration and the molar proportions of acetate, propionate, butyrate and the acetate: propionate ratio.

The findings of this study suggested that Sunphenon supplementation up to 40 g/kg DM of the diet could be a possible option to achieve optimum CH_4 reduction and to save dietary energy loss without any negative effect on whole-tract nutrient digestibility. Similarly the findings of our study on Euglena supplementation demonstrated that higher level of Euglena supplementation increased nutrient intake and CP retention without affecting nutrient digestibility. In general these studies illustrates that incorporation of natural feed additives such as Sunphenon 30S-O and Euglena in the ration of ruminants, have positive impact on reducing greenhouse gas emissions and improving efficiency of nutrient utilization for the potential benefit of the producers and sustainable environmental health.