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学　位　論　文　要　旨

畜産科学　専攻　博士後期　課程
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論文題目：Novel and Natural Feed Additive for Ruminants The Impacts on Behavior, Rumen Fermentation and Microbiome with special reference to Methane Emissions（反芻動物用の新規天然飼料添加物がメタン排出量、行動、ルーメン発酵、胃内微生物叢へ及ぼす影響）

要旨

The animal production industry is a significant source of GHGs. Ruminants represent the source of approximately 80% of the total GHG emissions from that sector. The most important greenhouse gas is CH4. The production of CH4 is formed mainly by enteric fermentation in the rumen. Emitted CH4 represents up to a 15% loss of feed energy. Numerous CH4 mitigation technologies have been investigated, with promising findings from dietary interventions. However, most of these strategies have only been investigated in vitro without being confirmed in vivo in addition to the adverse effects of some approaches on animal performance, rumen fermentation and behavior. Therefore, the main aim of this thesis was to provide a comprehensive evaluation of one of the novel and promising strategies (Mootral, a garlic and citrus extract) to mitigate CH4 missions from ruminants through in vitro and in vivo applications.

In Chapter 1, the first study was performed to understand Mootral's mode of action and to investigate its impacts on gas production, fermentation profile, and rumen microbiome. The experiment was a 24-h small-scale in vitro trial using rumen fluid collected from sheep. Mootral was supplemented in two dosages, 10% and 20% of the substrate (50% grass: 50% concentrate). Mootral impacts showed a dose-dependent effect on the investigated parameters. Supplementation with mootral led to increased production of total gas and total volatile fatty acids. Mootral supplementation reduced the proportion of CH4 in the produced gas by 22% and 54% for 10 and 20% Mootral, respectively. The fermentation profile was shifted toward more propionate and less acetate due to the addition of Mootral. The bacterial community showed an increase in the relative abundance of the Prevotellaceae family and Veillonellaceae family, while there was a decrease in the relative abundance of some hydrogen-producing bacteria by Mootral supplementation. The alteration in the archaeal community due to Mootral was obvious, where the Methanobacteriaceae family decreased and the Methanomassiliicoccaceae family increased in a dose-dependent manner. Thus, this preliminary study showed the ability of this novel combination to alter the rumen fermentation profile and reduce microbial groups associated with CH4 production, indicating the potential of this promising natural mixture to mitigate CH4 emissions from ruminants.

The aforementioned in vitro findings were further evaluated through an in vivo trial conducted in sheep, as described in Chapter 2, to confirm the potential of this new combination. The aim of this study was to determine the optimal effective dosage of Mootral for reducing CH4 production while considering its

impact on behavior, rumen fermentation characteristics, digestibility, and growth performance. The experiment was carried out using the same feeding style applied in vitro, while Mootral was evaluated at three dosages: 1, 5, and 10 g/kg DM, mixed thoroughly with concentrates and supplemented once daily in the morning feeding. The experimental design was a 4 × 4 Latin square design in which sheep were kept in individual metabolic cages. Mootral supplementation at all dosages had no adverse impact on feed intake, health status, growth performance, rumen fermentation, or nutrient digestibility. Mootral showed a dose-dependent manner in reducing CH4 production yield per digestible DM intake up to 12.8% with the highest dosage. The results of this study also indicated that Mootral supplementation improved animal welfare by reducing the incidence of abnormal behaviors. Therefore, it can be concluded that the natural combination of garlic and citrus has the potential to reduce CH4 emissions without any negative effects on animal health or behavior.

The experiment described in Chapter 3 was performed to investigate Mootral's potential to reduce CH4 production across different kinds of feeding styles (forage:concentrate ratios) in a different ruminant model: cows. The experiment was conducted as a batch culture for 24 h with rumen fluid collected from Holstein cows. Five experimental diets (forage:concentrate) were used: 100:0, 80:20, 60:40, 40:60, and 20:80. Mootral was supplemented at 200 g/kg of the substrate, which was selected based on the effective dosage from the first in vitro trial (Chapter 1). Each treatment had 6 replicates, and the experiment was repeated in three consecutive runs. The results of this trial confirmed the ability of Mootral to effectively reduce CH4 production for all tested feeding styles. The reduction potential per digestible DM was 44% in the high forage diet, and this reduction power increased with the inclusion of concentrate in the diet to reach up to 69% in the high concentrate diet. Mootral supplementation improved rumen fermentation by increasing the production of total volatile fatty acids and shifting the fermentation profile toward more propionate and less acetate. Additionally, Mootral had no adverse effects on nutrient digestibility. Thus, Mootral combination could work effectively as a CH4 inhibitor candidate in all feeding styles for ruminants.

備考　1　論文題目が英語の場合には，（　）書きで和訳を付す。

2　博士論文については，日本語の場合１８００～２２００字，英語の場合１０００～１４００語とする。修士論文については，それ以下でもかまわない。

3　図表は，要旨には記載しないこととする。

4　枚数は１枚を超えても差し支えない。