**Total tract digestibility and energy utilisation of phenotypically low and high methane intensity dairy cows**

**Application:** Long-term methane mitigation strategy by selecting cows with low methane intensity (g CH4 per kg of energy-corrected milk; ECM).

**Introduction:** Enteric CH4 from ruminant livestock contributes to 32% of global anthropogenic CH4 emissions (FAO, 2023) and represents a loss of gross energy intake of 6% on average (Niu et al., 2018). Therefore, reducing CH4 could theoretically improve animal performance, but recent knowledge suggests otherwise that phenotypically low enteric CH4 is related to less efficient fibre digestion. A promising strategy to mitigate CH4 is the selective breeding for low enteric CH4 intensity cows, i.e., cows low in enteric CH4 production per kg of milk or energy-corrected milk (ECM). In theory, efficient cows require less feed than average and can be expected to produce less CH4 per unit product at similar level of production (Waghorn and Hegarty, 2011). However, it is unclear whether divergence in CH4 intensity phenotype is indicative of efficiency in digestion and energy utilization, particularly because individual animals within a species and breed differ in CH4 production for reasons that cannot be explained by feed intake, the main determinant of CH4 production. Therefore, the current study aimed to evaluate the total tract digestibility and energy utilization between phenotypically low- and high- CH4-intensity dairy cows.

**Materials and Methods:** Sixteen lactating Norwegian Red dairy cows in their first to fourth lactation (9 primiparous and 7 multiparous) were used in a continuous experiment consisting of 11 days of adaptation and 6 days of data and sample collection (day 12 to 17), including total faces and urine collection for 3 days (day 15 to 17). Cows averaged (mean ± SD) 251 ± 30 days in milk, 638 ± 86 kg body weight, and 18 ± 4 kg of daily milk production at the beginning of the experiment. The experiment was conducted in two experimental runs with 8 animals per block. Cows were offered grass silage and concentrate at a proportion of 83:17 on a DM basis during the whole experiment. Feed intake and feed residues were recorded daily and sampled for wet chemical analysis. Gas exchange was estimated using GreenFeed (C-Lock, Inc., South Dakota, USA) over 6 days during data and sample collection with 5-6 hours interval to achieve all hours covering day and night measurement. Cows were milked daily, and samples were analysed for milk composition. According to the CH4 production expressed per unit of ECM in the experiment, cows were grouped into low- [LM; n = 8; CH4 intensity of 21±1 g CH4/kg ECM] and high- [HM; n = 8; 28 ± 1 g/kg] CH4 emitters. Linear mixed-effect model from the *nlme* package in R was used to test the differences between the two phenotypes on total tract nutrient digestibility and energy utilization. The model included phenotypes (LM and HM) as fixed effects and individual animal and parity (primiparous and multiparous) as random effects. Inclusion of parity as random factor in the model did not change effects and was therefore not included in the final model. Data are presented as Least Squares Means. Statistical significance of the fixed effect was stated if *P*<0.05, or tendency if 0.05≤*P*<0.10.

**Results:** Total-tract digestibility of DM, neutral detergent fibre (NDF) and acid detergent fibre (ADF) were not statistically different between groups (data not shown, P>0.1), while the digestibility of NDF and ADF were numerically higher in LM (74% and 71%, respectively) as compared with HM (72% and 69%). The gross energy intake, and energy loss in the faeces, urine, CH4, heat, and metabolizable energy intake were not different between the two phenotypes (P>0.1). However, LM cows were observed to retain more energy for milk production as compared to HM cows (66 and 49 MJ/d, P<0.05), while there was no difference in energy retention for body mass (P>0.1).

**Conclusions:** Differences in phenotypes could not be explained by differences in apparent nutrient digestibility but in energy utilization. Cows have similar metabolizable energy available, but cows with lower CH4 intensity utilize the energy to a greater extent for producing milk. Despite the metabolism of the host animal, phenotypes could potentially be explained by distinct microbiomes or microbial activity, whose analyses are underway.

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