

## Titanium dioxide as digestibility marker - Between and within cow variation in faecal concentrations

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Quantification of between and within cow variation in faecal nutrient concentrations is important when identifying the optimal sampling procedure for using digestibility markers in feeding trials. The aim of this experiment was to quantify the between cow variation in faecal concentration of titanium dioxide (TiO<sub>2</sub>), N and N:TiO<sub>2</sub> ratio, and to assess if within cow variation was affected by diet composition. Twelve Holstein cows were used in a 4 x 4 balanced Latin square with 4 diets, with 60:40 forage:concentrate ratio as dry matter (DM), and 4 periods of 21 days each. The 4 diets were 2x2 factorial arranged, including low vs high grass silage digestibility (LDGS vs HDGS) and 18 vs 42% of diet DM from maize silage (18MS vs 42MS). Faecal spot samples (300g) were collected twice daily from d 18 to 21 at h 08:00 and 14:00. Samples were analysed individually (8 samples/cow/period) for TiO<sub>2</sub> and N. Dependent variables (TiO<sub>2</sub>, N and N:TiO<sub>2</sub> ratio in faeces) were analysed in a linear mixed model including interaction between dietary factors and interactions between period, day and time as fixed effects, and cow and cow within period as random effects. Between cow variation was the standard deviation (SD) of the random cow effect from the model. Effect of diet on within cow variation was assessed using the raw data SD of cow within period (SD for the 8 samples/cow/period) for each given nutrient as dependent variable in a linear mixed model with interaction of dietary factors and period as fixed effects, and cow as random effect. Average and between cow variation (model mean ± SD for random cow) were 3.77±0.08 and 2.89±0.06 g/kg faecal DM for TiO<sub>2</sub> and N, and 0.77±0.03 g/g for N:TiO<sub>2</sub> ratio. Within cow variation was not affected by treatments, with the exception of faecal N SD, which tended to be higher for cows fed 42MS diets than cow fed 18MS diets (SD 0.28 vs 0.21 g/kg DM; P = 0.05). Within cow variation resulted in average and between cow variation of 0.36±0.06 and 0.25±0.03 g/kg faecal DM for TiO<sub>2</sub> and N, and 0.041±0.005 g/g for N:TiO<sub>2</sub> ratio. Eight spot samples were sufficient to have a stable within cow variation for TiO<sub>2</sub> and N:TiO<sub>2</sub> ratio, but high maize inclusion increased the within cow variation for faecal N concentration.