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**Comparison of enzymatic methods for estimating organic matter digestibility in forages**

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The precise and accurate estimation of organic matter digestibility (OMd) is essential for the nutritional evaluation of ruminant feed. Usually, in situ techniques are considered reference methods, but their application is limited by costs, time requirements and ethical concerns, highlighting the need for alternative approaches such as enzymatic methods. This study aimed to evaluate and compare different enzymatic methods for estimating OMd in forages, identifying reliable techniques suitable for routine laboratory analyses. Six forage samples (i.e., hay, grass silage, 3 sorghum silage, and maize silage) were analyzed using two enzymatic methods set for OMd evaluation: i) the French INRA method (Aufrère et al., 2007) and ii) the German Enzymatically Soluble Organic Matter (ELOS) method, derived from De Boever et al. (1986). In parallel, enzymatic methods developed by Gallo et al. (2017, 2018, 2019) were applied to determine the degradability of individual nutrients (NDF, protein and starch). For the latter methods, total tract OMd was estimated from digestible nutrient fractions, applying specific coefficients to predict post-ruminal digestion.A linear relationship was observed between INRA and ELOS estimates ($R^2$ = 0.845; RMSE = 3.65%), with ELOS values slightly overestimated OMd with respect to INRA. The OMd estimated from nutrient-specific enzymatic degradability showed relationships with both INRA ($R^2$ = 0.9914; RMSE = 1.93%) and ELOS ($R^2$ = 0.9956; RMSE = 2.58), confirming the robustness and consistency of here proposed nutrient-based estimation. Overall, the enzymatic estimation of OMd can be considered a valid alternative to in situ methods requiring use of fistulated animals, thus supporting the application of aforementioned methods in reliable and practical tool for routine forage evaluation.



**The role of rumen fluid adaptation: an in vitro comparison of diet-adapted and non–diet-adapted rumen fluid for continuous $CO_2$ and $CH_4$ monitoring with Gas Endeavour**

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In vitro gas production techniques, such as the Gas Endeavour system, are widely used to assess rumen fermentation kinetics, providing an effective tool to evaluate rumen function and possible methane mitigation strategies. Donor animal diet and forage characteristics shape rumen microbiota, fermentation, pH, microbial activity, and gas production in vitro. The study aimed to evaluate the effect of ruminal fluid (RF), adapted or non-adapted to the four experimental diets used in the in vivo trial, on in vitro fermentation parameters. All diets, formulated to have the same NDF and net energy, included 2000 g/head/d of hay, while varying concentrate and prickly pear peel silage: the control diet (CTR) contained 450 g/head/d of concentrate; diet A included 300 g/head/d of concentrate + 500 g/head/d of prickly pear peel silage; diet B included 150 g/head/d of concentrate + 1000 g/head/d of silage; diet C included 1500 g/head/d of silage. Diets (3 replicates) were incubated for 24 hours using the Gas Endeavour with two rumen inocula collected via an oro-esophageal probe: adapted rumen fluid (ARF) from 2 sheep fed each experimental diet, and common rumen fluid (CRF) from 2 sheep fed hay and concentrate, managed under identical conditions. A 2-way ANOVA (diet and RF as fixed factors) was used. Methane production in 24 h did not differ among diets, whereas total gas production was affected by both diet and RF: CTR diet produced the highest total gas, while ARF resulted in higher total gas production than CRF (276 vs. 267 mL/3g incubated feed). RF type influenced early gas production kinetics (first 7 h), after which curves converged. During this early phase, total gas and methane production were higher with CRF, whereas ARF reduced both parameters, particularly in diets B and C with highest silage inclusion. These findings show that dietary adaptation of the rumen microbiome significantly affects early fermentation, reducing total gas and methane production in initial stages, and highlight the necessity of using diet-adapted rumen fluid in in vitro studies for accurate and biologically meaningful results.