DNA Extraction from Equine Feces for Microbiome Studies – Optimization Challenges

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Introduction: The equine gut microbiome plays a key role in digestion and overall health, and fecal samples are commonly used for its analysis. However, extracting high-quality DNA from horse feces is challenging due to its fibrous content and presence of inhibitors. Optimizing extraction methods is essential to ensure reliable microbiome profiling. This study aimed to evaluate the performance of two DNA extraction protocols using a commercial kit and determine their suitability for microbiome analysis.

Material and Methods: Fresh feces were collected from adult horses. Samples were collected from the ground using sterile gloves and taken from the surface of the feces (without contact with the ground) and stored in sterile vials. The vials were kept refrigerated during transport to the laboratory, where they were frozen at -80 °C until processing. Samples used for DNA extraction were obtained from the center of the fecal ball.
Two DNA extraction methods were evaluated in this study. Method 1 involved directly weighing 20 mg of equine fecal material, which was processed using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Method 2 consisted of initial sample processing by diluting fecal samples 1:1 in sterile 0.05 M phosphate-buffered saline (PBS; pH 7.2), following the protocol described by other authors. The samples were homogenized and centrifuged to remove coarse particles. The resulting pellet was washed with PBS, centrifuged again, and then subjected to DNA extraction using the same QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany) following the manufacturer's protocol.
Extracted DNA concentration was measured using the Qubit Flex fluorometer (Invitrogen - Thermo Fischer Scientific, USA) with the dsDNA HS Assay Kit. DNA purity was assessed by measuring absorbance ratios (A260/A280 and A260/A230) using the Spex NanoSnap microvolume spectrophotometer (Cole-Parmer, New Jersey, USA).

Results: DNA extraction was performed using two different methodologies (Method 1 and Method 2). Both methods resulted in low DNA yields and poor purity. Using Method 1, all DNA concentrations were measured to be < 0.2 ng/µL by the Qubit flex. The purity ratios did not meet the recommended thresholds (OD260/280: 1.8–2.0; OD260/230: ≥ 1.8), indicating possible contamination or impurity, despite no visible signs of degradation or RNA contamination. Overall,

neither method produced DNA of sufficient quality or quantity for microbiome analysis.

Conclusions: The extraction methods tested were ineffective at obtaining amplifiable microbial DNA from horse feces. This underscores the necessity of protocol optimization or developing specialized methods tailored to equine fecal samples. Despite the lack of success, this study provides valuable insights into the limitations of the methodology and will help guide future research in equine microbiome studies.

Keywords: Equine feces, DNA extraction, Extraction method limitations, microbiome analysis.

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