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Research Article

Effect of Fecal Microbiota Transplantation on Insulinemia, Inflammation and Metabolic Markers in Horses with Equine Metabolic Syndrome

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Abstract | This study evaluated the effects of fecal microbiota transplantation (FMT) on insulinemia and inflammation in horses with Equine Metabolic Syndrome (EMS), in a context lacking access to microbiota sequencing. Ten EMS-affected Barb and Arab-Barb horses received two nasogastric FMT administrations from healthy donors (on Day 0 and Day 14), while ten EMS control horses received no treatment. Insulinemia, TNF-α, IL-6, and lipid profiles were monitored on Days 0, 7, 14, and 21. FMT significantly reduced plasma insulin (–29%), TNF-α (–24%), and IL-6 (–21%) by Day 14, with sustained effects through Day 21. Triglyceride levels also declined (~14%), while total cholesterol remained unchanged. These findings suggest that FMT may improve metabolic and inflammatory profiles in EMS-affected horses, even without microbiota sequencing. This low-cost intervention could represent a practical therapeutic strategy in resource-limited settings.

Keywords | Horse, Microbiota, Insulin, Inflammation, Fecal transplantation, Equine metabolic syndrome

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INTRODUCTION

Equine Metabolic Syndrome (EMS) is a complex endocrine disorder primarily characterized by insulin dysregulation, regional obesity, and a predisposition to laminitis (Durham *et al.*, 2019). This condition represents a major concern in equine veterinary medicine, both in terms of animal welfare and the economic burden of its management. Over the past decades, the gut microbiota has emerged as a key regulator of metabolic and immune functions across various species, including horses (Costa *et al.*, 2012; Fujisaka *et al.*, 2023).

Emerging evidence suggests that intestinal dysbiosis may contribute to EMS pathogenesis, as altered microbial composition is frequently associated with obesity and insulin resistance in horses (Mach *et al.*, 2017; Salem *et al.*, 2018). Alterations in the intestinal microbiota—known as dysbiosis—have been associated with several metabolic disorders, such as type 2 diabetes, obesity, and chronic inflammatory conditions in humans and various animal models (Le Chatelier *et al.*, 2013; DeGruttola *et al.*, 2016).

In this context, modulation of the microbiota—particularly through fecal microbiota transplantation (FMT)—has

garnered increasing interest as an innovative therapeutic strategy. In humans, FMT has shown promising results in the treatment of certain forms of diabetes and insulin resistance (Wu *et al*., 2023). Similarly, rodent studies demonstrated that microbiota transfer from lean donors improved insulin sensitivity and reduced systemic inflammation (Ridaura *et al*., 2013; Vassallo *et al*., 2025).

Although limited, pilot equine studies suggest that FMT can modulate the intestinal microbiota, improve digestive efficiency, and reduce inflammation (Schoster, 2018). In countries like Morocco, where access to next-generation sequencing technologies is limited, evaluating the impact of FMT through measurable biological markers—such as insulinemia and inflammatory cytokines—offers a pragmatic yet scientifically relevant alternative. This approach reflects a broader need for affordable microbiota-based therapies adaptable to low-resource environments.

Accordingly, this study evaluates whether fecal microbiota transplantation from healthy sport horses (KWPN) can improve changes in insulinemia and modulate inflammation in EMS-affected adult horses, without requiring direct sequencing of the microbiota. We hypothesize that FMT may improve insulin sensitivity and reduce systemic inflammation in EMS horses by indirectly modifying the gut microbiota, representing a feasible intervention in settings where omics technologies are not available.

MATERIALS AND METHODS

Animals and Study Design

Thirty adult horses were included and divided into three groups (n = 10 per group):

- Control group (Healthy Donors): KWPN horses (5–12 years) without digestive or metabolic disorders provided fresh feces for transplantation.
- EMS group: EMS horses of similar age and breed received no treatment.
- EMS + FMT group: Barb and Arab-Barb horses (10–18 years) diagnosed with EMS received fecal microbiota transplantation.

EMS diagnosis was based on biochemical (fasting insulinemia > 20 µIU/mL, insulin resistance confirmed by dynamic testing) and clinical criteria (BCS ≥ 7/9, history of laminitis) (Durham *et al*., 2019). All horses were kept under similar conditions (diet, housing, care).

All procedures were conducted in compliance with international veterinary ethical guidelines. Owner consent was obtained, and the protocol was supervised by a licensed equine veterinarian (Dr. Hannane Hmimia).

FMT Procedure

Fresh feces (1.2 kg) from donor horses were diluted in 4 L of warm sterile water (37–39 °C), homogenized for approximately 10 minutes, filtered to obtain a liquid fecal suspension, and administered via nasogastric tube to EMS-FMT recipients on Day 0 and Day 14. No adverse effects occurred following administration. Control horses received no FMT.

Sample Collection and Analysis

Blood samples were collected on Days 0, 7, 14, and 21. Insulin levels were measured using an equine-specific ELISA kit (Mercodia, Sweden). TNF-α and IL-6 concentrations were determined using ELISA kits (MyBioSource, USA). Triglycerides and total cholesterol were assessed with validated enzymatic kits (Spinreact, Spain). All samples were processed within 30 minutes of collection and stored at -80 °C.

Statistical Analysis

Data were analyzed using repeated measures ANOVA followed by post hoc tests. Significance was set at $p < 0.05$. Analyses were performed with SPSS v26.0.

Technical Limitations

Due to the lack of equine microbiota sequencing infrastructure in Morocco, direct sequencing of the microbiota was not possible. Instead, metabolic and inflammatory biomarkers were used as proxies to evaluate FMT effects. This approach is relevant in resource-limited settings lacking access to omics technologies.

RESULTS

Insulinemia

Figure 1A presents the impact of EMS and FMT on insulin levels over 21 days, demonstrating that the EMS group has continuously elevated insulin levels compared with the control group ($p < 0.001$). In contrast, the EMS + FMT group exhibits a significant decrease in insulinemia on day 21 compared with the EMS group ($p < 0.001$), approaching control levels and demonstrating a clear improvement in metabolic regulation; this suggests improved insulin sensitivity following FMT, likely linked to microbiota modulation.

Inflammatory Cytokines

Figures 1B and 1C show data on levels of inflammatory cytokines, specifically TNF-α (Figure 1B) and IL-6 (Figure 1C), in three experimental groups. In both Figures, the control group shows consistently low levels of inflammation, with TNF-α level of approximately 25-30 pg/mL and IL-6 of around 25 pg/mL throughout the study period. In contrast, when compared to the control group, the EMS group

showed markedly elevated levels of TNF-α ($p < 0.001$) and IL-6 ($p < 0.001$), starting high at day 0 and remaining significantly higher at day 21, indicating that EMS is linked to a strong and long-lasting inflammatory response. However, the EMS + FMT group showed a significant decrease in the levels of both cytokines at day 21 compared to the EMS group ($p < 0.001$), approaching those of the control group, indicating that modification of the gut microbiota by FMT may help restore immune balance and reduce EMS-related systemic inflammation (Figure 1).

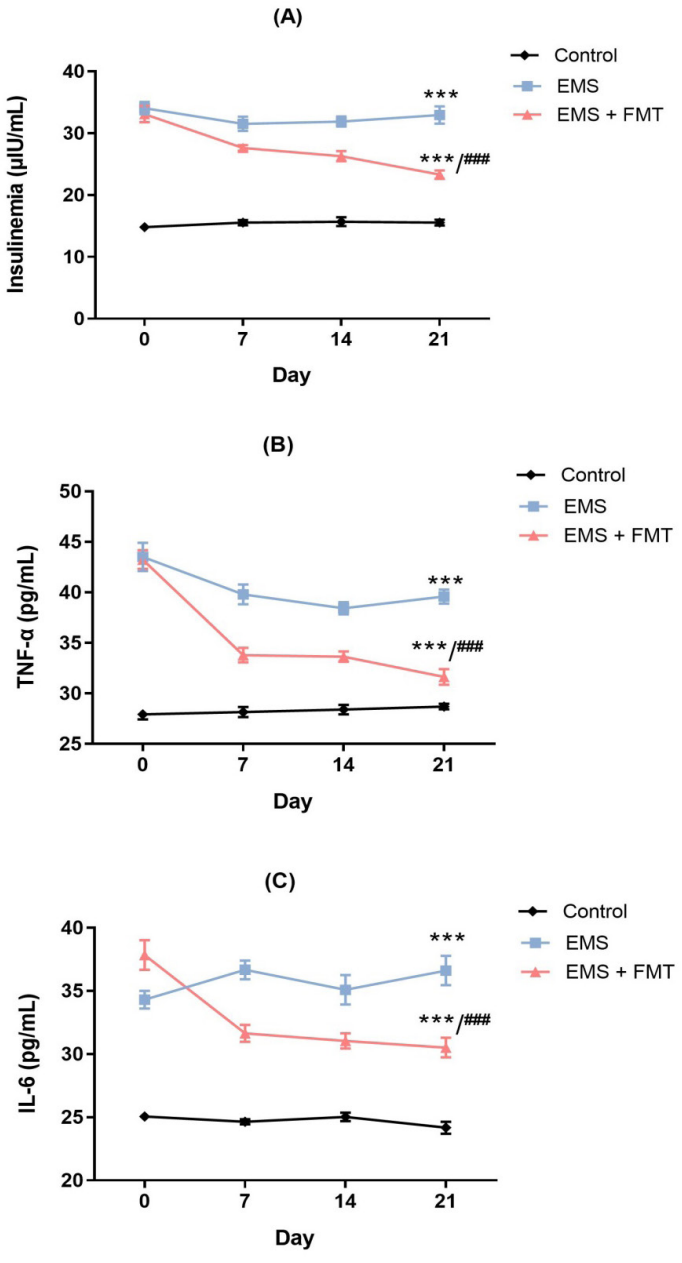

Figure 1: Impact of Fecal Microbiota Transplantation (FMT) on Insulinemia and Inflammatory Markers in Horses with Equine Metabolic Syndrome (EMS); **A:** Insulinemia (expressed in µIU/mL); **B:** TNF-α (expressed in pg/mL); **C:** IL-6 (expressed in pg/mL). Data are represented as mean ± S.E.M. *$p < 0.05$ vs. control group and #$p < 0.05$ vs. EMS group.

Lipid Profile

Figure 2 presents data on lipid metabolism, specifically triglycerides (Figure 2A) and cholesterol (Figure 2B), over 21 days in three experimental groups. In both Figures, the Control group shows stable and relatively low levels of triglycerides (~110 mg/dL) and cholesterol (~190 mg/dL) throughout the study. In contrast, the EMS group exhibits consistently elevated levels of both lipid markers as compared to the control group ($p < 0.001$), with triglycerides reaching ~150 mg/dL and cholesterol rising to nearly 230 mg/dL by Day 21. This indicates that EMS is linked to a significant dyslipidemia, characterized by increased circulating triglycerides and cholesterol. On the other hand, the EMS + FMT group shows a progressive decline in both triglycerides and cholesterol over time, approaching the levels observed in the Control group by Day 21 ($p > 0.05$) (Figure 2). These findings suggest that FMT may help restore normal lipid metabolism linked to EMS, potentially through modulation of gut microbiota and its downstream effects on host metabolism.

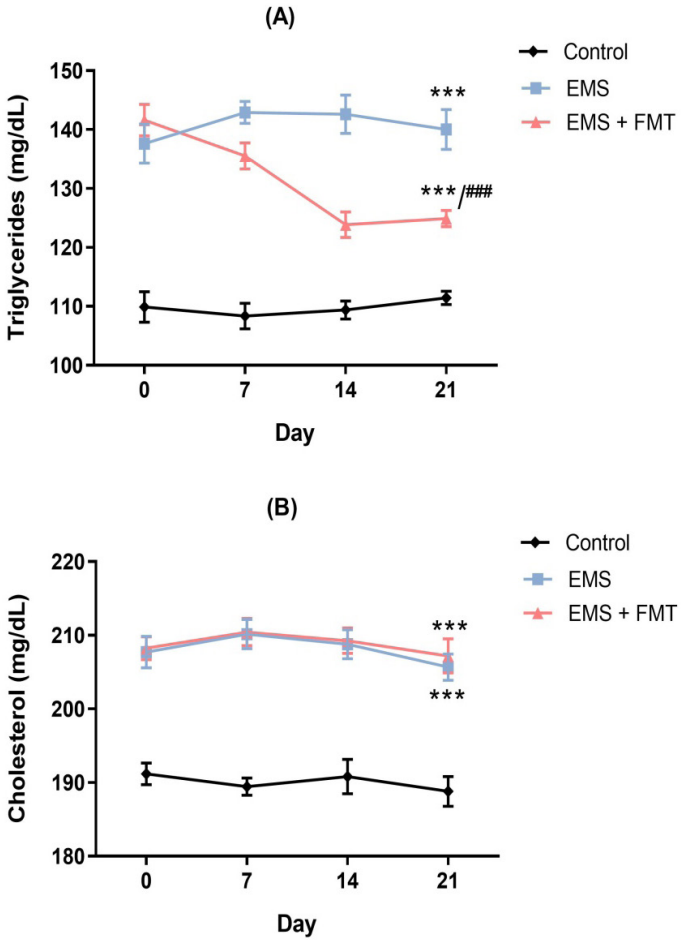

Figure 2: Impact of Fecal Microbiota Transplantation (FMT) on Lipid Markers in Horses with Equine Metabolic Syndrome (EMS); **A:** Triglyceride (expressed in pg/mL); **B:** Cholesterol (expressed in pg/mL). Data are represented as mean ± S.E.M. *$p < 0.05$ vs. control group and #$p < 0.05$ vs. EMS group.

DISCUSSION

In this study, FMT led to a significant reduction in insulinemia and inflammatory cytokines (TNF-α, IL-6) in EMS-affected horses by Day 14, with effects persisting until Day 21. Triglycerides also declined modestly, while total cholesterol remained stable. These outcomes align with previous equine studies showing microbiota interventions can improve digestive and immune function (Schoster, 2018). While comparisons with human trials offer mechanistic parallels, equine-specific validation of FMT protocols remains essential (Vrieze *et al*., 2012).

Advances in microbiota research have revealed its central role in metabolic regulation, particularly insulin sensitivity. FMT is emerging as a promising therapeutic approach for modulating glucose-insulin metabolism (Vassallo *et al*., 2025). In humans, studies have shown that FMT—alone or combined with metformin—can improve insulin resistance and body mass index by enhancing microbial diversity (Wu *et al*., 2023). A recent meta-analysis further confirmed its role in glycemic control and weight management (Hu *et al*., 2023). In the equine context, despite limited sequencing infrastructure for detailed microbiota analysis, our study provides preliminary evidence that FMT may improve insulin sensitivity and reduce inflammation in horses with EMS. The observed reduction in insulinemia supports its therapeutic potential.

The observed decrease in TNF-α and IL-6 is notable, as both are central mediators of systemic inflammation in EMS (Steelman *et al*., 2012). Similar anti-inflammatory effects have been reported in equine colitis, supporting the immunomodulatory potential of FMT (Schoster, 2018). However, the exact mechanisms remain speculative. The absence of direct sequencing of the microbiota is a key limitation, making it impossible to confirm shifts in bacterial populations. Future studies should include 16S rRNA gene sequencing or, in low-resource settings, targeted qPCR assays (e.g., for Akkermansia, Bacteroides, Clostridium).

FMT efficacy may also depend on the recipient's baseline microbiota. As shown in human studies, patients with marked dysbiosis benefit more from FMT (Zhang *et al*., 2013). It is plausible that EMS horses in this study had dysbiotic gut profiles, facilitating donor microbiota colonization. Another concern is the transient nature of the effects. While metabolic improvements persisted through Day 21, the short follow-up limits conclusions about long-term outcomes. Similar transience has been observed in horses when donor colonization is incomplete (Boucher *et al*., 2024). Larger, longer-term studies with stratification of responders are needed.

Interindividual variability was also evident, with some horses responding better than others. This may reflect differences in microbiota composition, immune function, or metabolic status, consistent with prior findings on equine gut microbiota diversity (Mach *et al*., 2017). To better understand microbiota–metabolism interactions, future trials should integrate clinical monitoring with microbial and metabolomic analyses, such as short-chain fatty acid profiling and targeted sequencing.

CONCLUSIONS AND RECOMMENDATIONS

In summary, our findings support the hypothesis that FMT may serve as a potential complementary treatment for EMS. Even without sequencing data, the observed reductions in insulin and inflammation are promising. This study contributes to the growing field of equine microbiome research and underscores the potential for FMT to be integrated into broader metabolic management strategies involving diet and exercise. Future studies should involve larger cohorts, longer follow-up, and microbial analyses (e.g., 16S rRNA sequencing or qPCR) to explore the underlying mechanisms of FMT. Evaluating the durability of clinical benefits over several months will be essential to validate this intervention in equine practice.

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NOVELTY STATEMENT

This study is original in demonstrating, for the first time, that FMT can significantly improve metabolic and inflammatory markers in horses with EMS without relying on microbiota sequencing. By showing the efficacy of a low-cost, accessible intervention in resource-limited settings, and by focusing on underrepresented breeds (Barb and Arab-Barb), the work offers a novel and practical therapeutic approach to managing EMS.

AUTHOR'S CONTRIBUTIONS

Hannane Hmimia: Conceptualization, funding acquisition, experimental design, fieldwork coordination, data collection, data interpretation, manuscript drafting, and critical revision.

Mouloud Lamtai: Statistical analysis, figure preparation, and assistance in data interpretation.

Aboubaker El Hessni and Abdelhalem Mesfioui: Supervision of the research project, critical revision, and final approval of the manuscript.

Highlights

- Fecal microbiota transplantation (FMT) significantly improved metabolic and inflammatory markers in horses with Equine Metabolic Syndrome (EMS).
- FMT significantly reduced insulin, TNF-α, IL-6, and triglyceride levels.
- FMT is considered a promising, low-cost therapeutic option for managing EMS.

Conflict of Interest

The authors declare no conflict of interest.

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