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**39 An in-Vitro Model of the Equine Fecal Microbiome to Assess How Horse Population Affects Fermentation Following a Starch**

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Abstract: The use of an in-vitro model of the equine microbiome is beneficial to assess how fermentation patterns differ based on a horse populations' habitual diet. The purpose of this study was to determine the in-vitro fermentation patterns of the microbial community within different horse populations before and following a starch challenge. Fecal samples were taken from three different populations of horses: horses from the Shackleford Banks, a North Carolina feral horse population living on native grasses; horses from the NCSU Equine Educational Unit that are predominantly kept on cool season mixed pastures, though may be supplemented with hay and concentrates when warranted; and privately owned horses that were fed mixed diets consisting of grass hay, concentrates and some pasture. Horses were monitored and fecal samples were collected immediately following a void and were stored on dry ice and frozen until analysis. Fecal samples from individual horses were pooled to form a representative sample for each population and mixed with an anaerobic medium to prepare an inoculum. The inoculum was placed into bottles containing either a treatment substrate of alfalfa (A) or of alfalfa and starch (AS). Bottles were capped, purged with CO<sub>2</sub> and placed in a water bath at 39°C to incubate for 0, 2, 4 or 24 hours. Culture samples were processed to measure methane and short chain fatty acids (SCFAs; acetate, propionate, butyrate and other isoacids) using gas chromatography. Results were analyzed using the Proc Mixed procedure in SAS to compare the effects of horse population, time and treatment. Methane was significantly greater after 24 hours within all populations with AS, as the inoculum compared with A alone ( $P = 0.03$ ). Propionate was greater for AS (molar percentage, mean  $\pm$  standard deviation;  $2.21 \pm 4.97\%$ ) versus the A treatment ( $11.95 \pm 4.97\%$ ,  $P = 0.02$ ). Acetate concentrations were significantly ( $P < 0.001$ ) greater within the A treatment in the Shackleford and NCSU horses ( $60.31 \pm 10.3\%$  and  $62.18 \pm 11.47\%$ , respectively) compared with the AS treatment ( $59.09 \pm 11.21\%$  and  $59.77 \pm 8.85\%$ , respectively). Privately owned horses showed similar values of acetate concentration when comparing the treatment of A ( $63.42 \pm 10.13\%$ ) versus AS ( $63.55 \pm 10.77\%$ ). Butyrate concentrations were greater in the

Shackleford Banks and NCSU horses ( $13.16 \pm 1.92\%$  and  $2.64 \pm 4.22\%$ , respectively) compared with the privately owned horses ( $10.96 \pm 2\%$ ). Isoacids were greater in the Shackleford Banks horses ( $10.19 \pm 4.15\%$ ) than the NCSU horses ( $9.87 \pm 3.98\%$ ) and the privately owned horses ( $9.52 \pm 3.53\%$ ;  $P < 0.0001$ ). It appears that fermentation of starch differs between these horse populations, likely due to their habitual diet.

**Keywords:** equine, fermentation, microbiome