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Fructooligosaccharide supplementation in the yearling horse: Effects on fecal pH, microbial content, and volatile fatty acid concentrations1,2

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ABSTRACT: Short-chain fructooligosaccharides (FOS) were supplemented to the diets of nine quarter horses ranging in age from 489 to 539 d with initial BW averaging 400.6 ± 21.2 kg. The objectives of this study were to determine the effects of dietary FOS on the fecal responses in terms of pH, the microbial population, and VFA concentrations. The horses were used in a 3 × 3 replicated Latin square design, fed according to NRC requirements, and their individual diets were supplemented with no FOS (CON), 8 g of FOS/d (LOW), or 24 g of FOS/d (HIGH) over three 10-d feeding periods. On the last 3 d of each 10-d feeding period, a single fecal sample was collected between 0730 and 0930. Fecal pH decreased linearly (P = 0.01) from 6.48 with the CON diet to 6.38 with the HIGH diet, but there was no change (P = 0.19 for linear effect) in fecal consistency among treatments. A quadratic effect (P < 0.01) was observed for fecal Escherichia coli population, but no difference (P = 0.88 for linear effect) was found in fecal Lactobacilli enumeration among treatments. The presence of fecal Bifidobacteria was unable to be confirmed and was therefore not reported. Fecal acetate concentrations increased linearly (P = 0.03), with means of 2.13, 2.18, and 2.52 mg/g of wet feces for CON, LOW, and HIGH treatments, respectively. Similarly, fecal propionate concentrations increased linearly (P = 0.01), with means of 0.58, 0.64, and 0.73 mg/g for CON, LOW, and HIGH treatments, respectively. Fecal butyrate concentrations also increased linearly (P = 0.02), with means of 0.40, 0.46, and 0.54 mg/g for CON, LOW, and HIGH treatments, respectively. Total VFA (P = 0.01) and lactate (P = 0.02) concentrations increased linearly, with total VFA means of 3.47, 3.69, and 4.25 mg/g for CON, LOW, and HIGH treatments, respectively, and lactate means of 0.36, 0.41, and 0.47 mg/g for CON, LOW, and HIGH treatments, respectively. Supplementing FOS in diets fed to yearling horses altered fecal microbial populations, fecal VFA concentrations, and pH.

Key Words: Fecal, Fructooligosaccharide, Horse, Microbiology, Volatile Fatty Acids


Introduction

Fructooligosaccharides (FOS) are naturally occurring short- and medium-chain fructose molecules linked by a β 2-1 glycosidic bond (Gibson and Wang, 1994a). The β 2-1 bond prevents these polysaccharides from being hydrolyzed by mammalian digestive enzymes; thus, they are fermented predominantly in the hind gut by microorganisms (Roberfroid, 1997). In the large intestine of humans, rats, and pigs, short-chain FOS have been found to stimulate production of beneficial bacteria such as Bifidobacteria (Gibson and Roberfroid, 1995; Howard et al., 1995; Campbell et al., 1997). These bacteria can hydrolyze β 2-1 bonds and are thereby able to use FOS as an energy source. The growth of Bifidobacteria has been shown to increase the production of VFA and lactic acid, which lowers pH in the large intestine and inhibits the growth of pathogenic bacteria (Gibson and Wang, 1994b; Campbell et al., 1997).

Measurement of cecal or colonic responses, such as pH, microflora, short-chain fatty acid concentration, and epithelial cell proliferation, have been used to determine gut health in a number of species (Howard et al., 1995; Campbell et al., 1997; Garret et al., 2002). Fecal responses of pH, microflora, and color also have been used to assess colonic health in rats, pigs, and humans (Benno and Mitsuoka, 1992; Howard et al., 1995; Campbell et al., 1997). Maintenance of colonic health in the horse is essential to avoid disorders such as colic, equine dysautonomia, or laminitis. Equine intestinal disorders in the United States (from spring...
Table 1. Composition of concentrate fed to yearlings at 1% BW daily

<table>
<thead>
<tr>
<th>Item</th>
<th>% as-fed basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient</td>
<td></td>
</tr>
<tr>
<td>Cracked corn</td>
<td>37.40</td>
</tr>
<tr>
<td>Whole oats</td>
<td>40.90</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>9.00</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>5.00</td>
</tr>
<tr>
<td>Trace mineral salt</td>
<td>0.50</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.70</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.70</td>
</tr>
<tr>
<td>Vitamin A/D/E premix(^a)</td>
<td>0.80</td>
</tr>
<tr>
<td>Molasses</td>
<td>5.00</td>
</tr>
</tbody>
</table>

Analyzed composition

- Moisture, %: 12.24
- Fat, %: 3.93
- CP, %: 13.44
- Ca, %: 0.61
- P, %: 0.56

\(^a\) Vitamin premix provided per kilogram of grain concentrate: vitamin A, 5,000 IU; vitamin D\(_3\), 408 IU; vitamin E, 132 IU.

1998 to spring 1999) had an estimated cost of $115,300,000 (Traub-Dargatz et al., 2001). Improving gut health in the horse has the potential to decrease this cost. Therefore, the objective of the present study was to compare the effects of two levels of FOS on the fecal responses of pH, microbial content, and VFA concentrations as indicators of intestinal health of yearling horses.

Materials and Methods

Management of Animals

Nine quarter horses (six geldings and three fillies) ranging in age from 489 to 539 d with initial BW averaging 400.6 ± 21.2 kg were used for this experiment. Horses were kept on an 8.1-ha orchardgrass pasture at the University of Missouri Horse Teaching and Research Facility and had ad libitum access to pasture grass, fresh water, and a plain salt block throughout the study. Horses were individually fed a concentrate supplement (Table 1) at 1% of their BW on an as-fed basis according to NRC requirements (1989) for long yearlings not in training, with the remaining requirements being met from pasture. This was a 30-d study conducted during September and October. The amount of concentrate fed was split equally between a morning (0730) and afternoon (1630) feeding, with the FOS being added daily to the morning feeding. The yearlings not in training, with the remaining treatments. The HIGH treatment was then added. The trial consisted of three consecutive 10-d feeding periods. The horses were blocked by BW into three groups of three horses, with one horse from each group receiving a different treatment during each 10-d feeding period, such that all horses received all treatments.

Sample Collection

Horses were given 7 d to acclimate to the diet and single fecal samples were collected from the surface of rubber-matted stall floors from each horse (to fill a 250-mL container) immediately after defecation during the 0730 feeding on the last 3 d of each 10-d feeding period. Horses routinely defecated within 2 h of being brought into stalls.

Fecal pH

Fecal pH was determined immediately after defecation by thoroughly mixing equal amounts of feces and double-deionized water in 50-mL tubes and submerging the pH probe (model 115, general purpose combination glass probe; Corning Science Products, Corning, NY) in the mixture until the reading stabilized. The pH meter was calibrated immediately before data collection.

Microbial and VFA Analyses

For later analysis of Lactobacilli, Escherichia coli, and Bifidobacteria populations, approximately 10 g of feces from the single fecal sample was mixed with 10 mL of an autoclaved glycerol salt solution (FDA, 1998), placed on ice, and then frozen at −80°C until further processing. Fecal samples were thawed in an anaerobic chamber and serially diluted in anaerobic diluent (Bryant and Burkey, 1953). Three dilutions were inoculated onto different plates to maximize counting precision.

For Lactobacillus, samples were diluted from 10\(^{-4}\) to 10\(^{-6}\), and for Bifidobacteria and E. coli, samples were diluted from 10\(^{-5}\) to 10\(^{-7}\). The media for Lactobacillus bacteria was anaerobic de Man, Rogosa, and Sharpe (MRS) broth (#288130; Difco, Sparks, MD) and agar (20 g/L), with 20 mg/L vancomycin supplement and bromocresol green (LAMVAB; Hartemink et al., 1997). The media for Bifidobacteria was a modified Columbia agar, containing 0.5% (vol/vol) propionic acid and ad-
justed to a pH of 5.0 (Beerens, 1990). This medium is both elective and selective for Bifidobacteria. Confirmation of Bifidobacteria was by fructose-6-phosphate phosphoketolase test (Scardovi, 1986). Incubation time for Lactobacilli and Bifidobacteria was 48 h at 37°C in an anaerobic gas chamber (Model 1025; Forma Scientific, Inc., Marietta, OH) under 85% N2, 10% H2, and 5% CO2. The E. coli was enumerated (cfu/mL) by a Petrifilm method according to the manufacturer’s instruction (Petrifilm; 3M, St. Paul, MN). Fecal contents were measured for concentrations of acetate, butyrate, propionate, and lactate by gas chromatography (Varian Model 3400; Varian Instrument Group, Walnut Creek, CA; Goetsch and Galyean, 1983).

Fecal Consistency. Fecal consistency was recorded on collection days and subjectively scored on a scale from 1 to 5, with 1 being extremely dry, 3 being normal, and 5 being watery diarrhea. This scale was assigned by the authors, and fecal consistency was ranked by the same individual throughout the duration of the study.

Statistical Analyses. Data were analyzed by ANOVA using the PROC GLM procedures of SAS (SAS Inst., Inc., Cary, NC). The statistical model included the effects of treatment CON (0), LOW (8), and HIGH (24), period (I, II, or III), square, and horse within square. The effect of horse contained 8 df in the ANOVA. This 8 df came from the variation due to square (three Latin squares) plus horse within square. Linear and quadratic effects were tested in the form of contrasts for unequally spaced treatments. An alpha level of 0.05 was used for determination of statistical significance.

Results

Fecal pH decreased linearly (P = 0.01) as the amount of FOS supplemented in the diet increased (Table 2). Fecal consistency was normal and did not differ (P = 0.19 for linear effect) among treatments throughout the study (Table 2). A quadratic effect (P < 0.01) was observed for fecal E. coli population, but no difference (P = 0.88 for linear effect; P = 0.29 for quadratic effect) was found in fecal Lactobacilli enumeration among treatments (Table 2). Presence of fecal Bifidobacteria was unable to be confirmed by fructose-6-phosphate phosphoketolase test (Scardovi, 1986); therefore, enumeration of Bifidobacteria is not reported. A linear increase was demonstrated in concentrations of fecal lactate (P = 0.02), acetate (P = 0.03), butyrate (P = 0.02), propionate (P = 0.01), and total VFA (P = 0.01) as the quantity of FOS supplemented increased (Table 2).

Discussion

In this study, increased supplementation of FOS resulted in decreased fecal pH. This drop in pH would be expected as production of lactic acid and short-chain fatty acids in the gut increased. Campbell et al. (1997) demonstrated lowered fecal pH in rats supplemented with FOS, which agrees with our findings. In contrast, studies in other species have failed to show a difference in fecal and/or cecal pH as a result of FOS supplementation (Howard et al., 1995; Bouhnik et al., 1999; Hunter et al., 1999). This lack of change in pH could be a result of different intestinal microbial environments among species. Because some bacterial populations (e.g., Bifidobacteria) utilize FOS more readily than others (Gibson and Wang, 1994a), the production of VFA and lactate in the gut varies. As a result, fecal pH also may be affected, as was the case in the present study. A similar pattern of decreased fecal pH coupled with increased fecal VFA and lactate concentrations was demonstrated in a study by Hussein et al. (2004) as grains were added to a base diet of alfalfa cubes fed to horses. The fecal VFA concentrations reported by Hussein et al. (2004) for horses fed the control diet of alfalfa cubes were similar to the fecal VFA concentrations in this study.

Excess dietary intake of carbohydrates by the horse results in starch fermentation in the hindgut, leading to increased lactate production and consequently low

Table 2. Effect of fructooligosaccharide (FOS) on fecal responses in yearling horsesa,b

<table>
<thead>
<tr>
<th>Item</th>
<th>FOS, g/d</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>pH</td>
<td>6.48</td>
<td>6.44</td>
</tr>
<tr>
<td>Consistencyc</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Escherichia coli, log10 population</td>
<td>4.90</td>
<td>4.75</td>
</tr>
<tr>
<td>Lactobacilli, log10 population</td>
<td>7.11</td>
<td>7.06</td>
</tr>
<tr>
<td>Lactate, mg/gd</td>
<td>0.36</td>
<td>0.41</td>
</tr>
<tr>
<td>Acetate, mg/gd</td>
<td>2.13</td>
<td>2.18</td>
</tr>
<tr>
<td>Propionate, mg/gd</td>
<td>0.58</td>
<td>0.64</td>
</tr>
<tr>
<td>Butyrate, mg/gd</td>
<td>0.40</td>
<td>0.46</td>
</tr>
<tr>
<td>Total VFA, mg/gd</td>
<td>3.47</td>
<td>3.69</td>
</tr>
</tbody>
</table>

aEach mean represents nine individually supplemented horses.
bPresence of Bifidobacteria was unable to be confirmed and therefore enumeration was not reported.
cFecal consistency was subjectively scored on a scale from 1 to 5, with 1 being extremely dry, 3 being normal, and 5 being watery diarrhea.
dData are on a wet basis.
and potentially detrimental cecal pH values (Garner et al., 1978; Bailey et al., 2002). This may contribute to bouts of colic (Reeves et al., 1996) and laminitis (Garner et al., 1978; Bailey et al., 2002) in the horse. In the current study, we did not collect cecal samples; therefore, we were unable to determine whether a correlation between cecal and fecal pH existed. A correlation between cecal and fecal pH has been reported in rats with fecal pH consistently being greater than cecal pH (Campbell et al., 1997). In the present study, the decrease in fecal pH observed as a result of FOS supplementation caused no deleterious effects on any of the horses during the study or after its completion.

Fecal acetate, butyrate, propionate, lactate, and total fecal VFA concentrations all increased as the quantity of FOS supplemented in the diet increased. This finding suggests that by providing FOS as an energy source for bacterial populations, VFA production increased, resulting in additional energy available for the horse. Our findings are in contrast with those of Campbell et al. (1997), who found no difference in fecal short-chain fatty acid concentrations of rats fed FOS vs. those fed the control diet. Campbell et al. (1997) also found that cecal VFA concentrations were not correlated with fecal VFA concentrations; however, fecal VFA concentrations have served as indicators of fermentation patterns in the large intestine of dairy cows (Meylan et al., 2002). Although fecal VFA concentrations may not represent actual cecal VFA concentrations, fecal VFA concentrations can provide relevant information regarding increases or decreases in VFA production, as was demonstrated in the present study.

It has been shown in humans, rats, and pigs that competitive exclusion of harmful bacteria by beneficial bacteria has the potential to improve intestinal health (Gibson and Roberfroid, 1995; Howard et al., 1995; Campbell et al., 1997). An increase in the population of pathogenic bacteria has been associated with enterocolitis, severe diarrhea, and equine dysautonomia (East et al., 2000; Weese et al., 2001; Garrett et al., 2002). In the present study, the fecal population of *Lactobacilli* was not altered as a result of FOS supplementation, but the number of *E. coli* was decreased in horses supplemented with 8 g of FOS daily; however, fecal *E. coli* numbers for the HIGH horses did not differ from those of the control group. This finding may indicate that the optimal dose of FOS supplementation needed to decrease *E. coli* population in these yearlings was between 8 and 24 g. Campbell et al. (1997) also reported no difference in fecal *Lactobacilli* population between control rats and those supplemented with FOS, but they reported an increased fecal *Bifidobacteria* population, as well as overall anaerobes. Bouhnik et al. (1999) reported a significant correlation between the dose of FOS ingested and fecal *Bifidobacteria* counts in healthy humans. Increased population of *Bifidobacteria* are often reported in species supplemented with FOS or other oligosaccharides, as this species of bacteria is able to efficiently utilize FOS as an energy source (Howard et al., 1995; Campbell et al., 1997; Bouhnik et al., 1999). Because we were unable to confirm the presence of *Bifidobacteria* in this study, we can neither refute nor substantiate the relationship of *E. coli* to *Bifidobacteria*. Additionally, because a limited number of species were measured, important information on fecal bacterial content is lacking. Research is needed to evaluate a broader array of microbial species to provide a more complete understanding of the fecal microbial environment and how it is affected by FOS supplementation. It also should be noted that fecal bacterial populations may not represent what has colonized the intestine, but rather what has passively moved through the intestine (Weese et al., 2003). Although the result of the present study demonstrated an effect of FOS on fecal *E. coli* population, whether this is representative of cecal microbial populations requires further investigation.

**Implications**

We demonstrated that fructooligosaccharide supplemented to equine diets decreased fecal *Escherichia coli* populations when supplemented to the diet at the rate of 0.02 g/kg of BW, and it improved the energy status of horses, as evidenced by increased production of fecal volatile fatty acids. Therefore, the effects of fructooligosaccharide on gut health in the horse merit further investigation. Improving the gastrointestinal health of the horse can improve equine well-being and decrease economic loss incurred by the equine producer. Additional research must be done to characterize the bacterial population of the equine intestine to achieve a more complete understanding of normal gut microflora populations in the horse. Elucidating the relationship between fecal and cecal microbial populations, pH, and volatile fatty acid concentrations also is of importance to determine the accuracy of fecal responses as indicators of gut health in the equine.

**Literature Cited**


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