Microbiote activity and immunity of horses fed with scFOS and subjected to a vaccination

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What are short-chain fructooligosaccharides?

- Prebiotic fibres obtained from sugar beet, by a bioenzymatic reaction:

- EU Regulation 68/2013, scFOS = raw material (n° 4.1.14)

G: Glucose; F: Fructose
In horses, scFOS modulate microbiota from stomach to colon

Pelligrini et al., 1999; Berg et al., 2005; Respondek et al., 2007 and 2008.
Microbiota is known to be involved in mammal’s immune response.

- scFOS
- Gut microbiota
- Immune response

In pigs, calves, mice, and horse.

Maslowski and Mackay, 2011
Hooper et al., 2012
Maynard et al., 2012
Kamada et al., 2013
Kato et al., 2014, …
ScFOS modulate immune response after a vaccine challenge in piglets

IgA response to influenza vaccination in serum and faeces

# Tendency to be different, 0.05 < p ≤ 0.1.
* Significantly different, p ≤ 0.05.

p=0.002
R²= 0.31
Objectives of the study

- To measure the potential changes of
  - the intestinal ecosystem activity
  - the immune response
  of horses subjected to an EHV1-EHV4 vaccination and fed or not with scFOS

- To establish potential correlations between local and systemic parameters
Horses and horse management

- Trial conducted in horse facility d’AgroSup Dijon
- 8 crossbred gelding
  - 12.9 ± 3.3 years
  - BW: 487 ± 33 kg
  - Body condition score: 3.4
  - Cannulas in the caecum and right ventral colon

- Individual free-stalls (13.3 m², wood shavings)

- Exercise
  - 1 hour/day (except on days of measurements)
Experimental design

Adaptation to the basal diet (70:30 hay: grain ratio, 1.7% BW) for 3 weeks.

CONTROL DIET: 30 g/day maltodextrines

scFOS DIET: 30g/day Profeed®

D0: Baseline
D-1: Adaptation to basal diet
D21: Vaccination against EHV1-4
D36: Intestine, saliva, blood sampling
D43: Intestine, saliva, blood sampling
Measures and analysis

- Caecal and colonic pH and VFA
- tIgA in serum, saliva, intestinal filtrates
- Blood immune cells, specific Ig

Statistical analysis:

- ANCOVA:
  - D-1 as covariable, Treatment and day effect + their interaction as fixed factor, Horse: randomized effect
- PCA for D-1, D21, D36 and D43
1. Vaccination: changing digestive parameters

- Vaccination and immune response result in modifying fermentative orientations in horses: new observation

### D-lactate, mmol/L

<table>
<thead>
<tr>
<th>Time</th>
<th>Caecum</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>b</td>
<td>a</td>
</tr>
<tr>
<td>36</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>43</td>
<td>a</td>
<td>a</td>
</tr>
</tbody>
</table>

### Butyrate, mmol/L

<table>
<thead>
<tr>
<th>Time</th>
<th>Caecum</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>36</td>
<td>b</td>
<td>a</td>
</tr>
<tr>
<td>43</td>
<td>a</td>
<td>a</td>
</tr>
</tbody>
</table>

Caecum: $P < 0.0001$
Colon: $P = 0.01$

$P = 0.05$
1. Vaccination: changing innate and specific immune response within 2 weeks

Consistent with Goundasheva et al., 2005

Rather weak specific response
1. Vaccination: changing local immune response...

Fluxes between intestine and blood?
2. scFOS: no modulation of intestine ecosystem activity and immune parameters

No effect on ecosystem activity: P > 0.05 for VFA and pH in caecum and colon (data not shown).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>scFOS</th>
<th>SEM</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>21</td>
<td>36</td>
<td>43</td>
<td>21</td>
</tr>
<tr>
<td>tlgA-Saliva (pOD)</td>
<td>14.5</td>
<td>42.8</td>
<td>16.1</td>
<td>22.5</td>
</tr>
<tr>
<td>tlgA-Caecum (pOD)</td>
<td>72.2</td>
<td>43.0</td>
<td>17.0</td>
<td>56.0</td>
</tr>
<tr>
<td>tlgA-Colon (pOD)</td>
<td>78.7</td>
<td>48.2</td>
<td>32.8</td>
<td>42.1</td>
</tr>
</tbody>
</table>
2. scFOS: increasing serum tlgA

In agreement with studies using mice, humans, calves (Pierre et al., 1997; Manhart et al., 2003; Tai et al., 2009)
2. scFOS: decreased horse inter-variability before vaccination

No cluster before scFOS supplementation and after vaccination

In agreement with Respondek et al., 2008

- High concentration of caecal VFA (61.3±7.82 mmol/L)
- High percentage of colonic acetate (73.0±1.5%)
- High concentration of serum tlgA (55.4±12.5 pOD)
Conclusions

- First study in horse to observe simultaneously
  - local and systemic immune parameters
  - Immune and fermentative parameters

- Vaccination results in changes of both immune and digestive parameters
  - Link between microbiota and local/systemic immunity?
  - Fluxes between different parts of the body?
Conclusions

- scFOS modulate immune parameters of horses notably by increasing tlgA.
  - In agreement with other studies in other species

- scFOS reduce inter-variability between horses before vaccination but this effect disappears after vaccination
  - Benefits?
  - Correlations between fermentative and immune parameters?
Thank you for your attention!

Questions?

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Biochemical composition of hay and pelleted complementary feed, % of DM

<table>
<thead>
<tr>
<th></th>
<th>Grass Hay</th>
<th>Pelleted complementary feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic Matter</td>
<td>91.5</td>
<td>93.1</td>
</tr>
<tr>
<td>Neutral Detergent Fiber</td>
<td>63.4</td>
<td>48.2</td>
</tr>
<tr>
<td>Acid Detergent Fiber</td>
<td>33.4</td>
<td>22.1</td>
</tr>
<tr>
<td>Acid Detergent Lignin</td>
<td>3.4</td>
<td>4.1</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>7.6</td>
<td>14.0</td>
</tr>
<tr>
<td>Starch</td>
<td>ND⁷</td>
<td>18.0</td>
</tr>
<tr>
<td>Fat</td>
<td>ND⁷</td>
<td>3.5</td>
</tr>
</tbody>
</table>
# Digestive parameters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>scFOS</th>
<th>P values$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day (D) ♦</strong></td>
<td>21 36 43</td>
<td>21 36 43</td>
<td>SEM$^1$</td>
</tr>
<tr>
<td><strong>Caecum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total VFA</strong></td>
<td>46.5 69.9 50.1</td>
<td>62.7 66.9 53.2</td>
<td>6.08</td>
</tr>
<tr>
<td><strong>Acetate</strong></td>
<td>33.5 50.0 35.9</td>
<td>45.7 47.1 36.8</td>
<td>4.50</td>
</tr>
<tr>
<td><strong>Propionate</strong></td>
<td>9.9 14.5 10.3</td>
<td>12.7 13.7 11.2</td>
<td>1.57</td>
</tr>
<tr>
<td><strong>Butyrate</strong></td>
<td>2.5 3.9 3.0</td>
<td>3.8 4.0 3.4</td>
<td>0.55</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>6.8 6.6 6.5</td>
<td>6.7 6.6 6.6</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>L-lactate</strong></td>
<td>1.42 0.88 0.03</td>
<td>0.34 0.92 0.32</td>
<td>0.687</td>
</tr>
<tr>
<td><strong>D-lactate</strong></td>
<td>2.69 0.42 ND</td>
<td>1.92 0.38 0.07</td>
<td>0.346</td>
</tr>
<tr>
<td><strong>Colon</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total VFA</strong></td>
<td>60.0 62.8 51.6</td>
<td>64.1 60.1 56.5</td>
<td>5.20</td>
</tr>
<tr>
<td><strong>Acetate</strong></td>
<td>40.8 42.1 35.1</td>
<td>47.9 42.8 40.3</td>
<td>3.61</td>
</tr>
<tr>
<td><strong>Propionate</strong></td>
<td>12.7 14.6 10.6</td>
<td>12.4 13.3 11.5</td>
<td>1.12</td>
</tr>
<tr>
<td><strong>Butyrate</strong></td>
<td>4.0 3.7 3.6</td>
<td>3.9 3.4 3.6</td>
<td>0.53</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>6.9 6.6 6.5</td>
<td>6.6 6.6 6.6</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>L-lactate</strong></td>
<td>1.2 1.44 1.04</td>
<td>0.76 1.81 1.75</td>
<td>0.600</td>
</tr>
<tr>
<td><strong>D-lactate</strong></td>
<td>3.13 0.36 ND</td>
<td>3.27 2.38 1.11</td>
<td>0.700</td>
</tr>
</tbody>
</table>
This effect was not observed after vaccination

Day 36

Day 43

No such cluster on Day 36 and 43:
- Immune response variability?
- Vaccine effect easier to describe than the scFOS effect?