Impact of antibiotherapy on growing rabbits assessed by a whole-blood transcriptomic approach

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INTRODUCTION

Reduction of antibiotic use

(societal expectations and European pressure)

Without degrading performances of rabbits (growth and health)

3 different approaches

- Phenotypic
- Transcriptomic
- Metagenomic
MATERIALS AND METHODS
Materials and Methods

Phenotypic study

Control diet
n = 150

Antibiotics diet
n = 150

- Tiamulin (100 ppm)
- Apramycin (32.5 ppm)

D0 D15 D29 D45 D60 D70

Weaning

Solid feed

Milk

n = 150

n = 150

daily: mortality + morbidity
weekly: weight of rabbits + feed intake

Slaugther: sampling of blood tissues, feces, and cecal content

VINCENT JACQUIER / IMPACT OF ANTIBIOTHERAPY ON RABBITS AND WHOLE-BLOOD TRANSCRIPTOMIC APPROACH
Materials and Methods

Transcriptomic study

CRB GADIE (http://crb-gadie.inra.fr/)

Blood

\[ \text{D}_{29} \quad \text{D}_{45} \]

RNA extraction

RT, labeling, Hybridization, and scan

Statistical analysis (R – Limma)

Ingenuity Pathway Analysis (IPA)

2 groups

\[ \times \quad 2 \text{ ages} \]

\[ \times \quad 8 \text{ animals} \]

= 32 samples

etc.
RESULTS
Results

Phenotypic study – Feed Intake

<table>
<thead>
<tr>
<th>Period</th>
<th>AB</th>
<th>C</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed Intake (g/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$D_{28-42}$</td>
<td>104.6</td>
<td>97.9</td>
<td>2.3</td>
<td>0.16</td>
</tr>
<tr>
<td>$D_{42-70}$</td>
<td>153.5</td>
<td>151.1</td>
<td>3.0</td>
<td>0.69</td>
</tr>
<tr>
<td>$D_{28-70}$</td>
<td>137.2</td>
<td>133.3</td>
<td>2.3</td>
<td>0.42</td>
</tr>
</tbody>
</table>

No impact of antibiotherapy on the feed intake & feed conversion ratio

<table>
<thead>
<tr>
<th>Period</th>
<th>AB</th>
<th>C</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed Conversion Ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$D_{28-42}$</td>
<td>1.85</td>
<td>1.88</td>
<td>0.02</td>
<td>0.61</td>
</tr>
<tr>
<td>$D_{42-70}$</td>
<td>3.30</td>
<td>3.43</td>
<td>0.06</td>
<td>0.32</td>
</tr>
<tr>
<td>$D_{28-70}$</td>
<td>2.77</td>
<td>2.85</td>
<td>0.04</td>
<td>0.31</td>
</tr>
</tbody>
</table>
Results

Phenotypic study – Weight

<table>
<thead>
<tr>
<th>Period</th>
<th>AB</th>
<th>C</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D_{28-42}</td>
<td>56.5</td>
<td>52.3</td>
<td>1.1</td>
<td>0.047</td>
</tr>
<tr>
<td>D_{42-70}</td>
<td>46.4</td>
<td>44.1</td>
<td>0.6</td>
<td>0.075</td>
</tr>
<tr>
<td>D_{28-70}</td>
<td>49.6</td>
<td>46.8</td>
<td>0.6</td>
<td>0.014</td>
</tr>
</tbody>
</table>

And a greater weight gain

+ 130 g / rabbit
For AB group
Results

Phenotypic study – Health Status

- Globally: good health status
- A post weaning morbidity rate lower with AB diet
- But not between 42-70d

And...

- No significant difference in mortality rate (P=0.32)!

<table>
<thead>
<tr>
<th>Period</th>
<th>AB</th>
<th>C</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>28-42d</td>
<td>0.9</td>
<td>10.9</td>
<td>0.002</td>
</tr>
<tr>
<td>42-70d</td>
<td>2.6</td>
<td>3.0</td>
<td>1</td>
</tr>
</tbody>
</table>

Morbidity rate (%)
## Results

Transcriptomic study

### Differential expression (DE)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Probes DE</th>
<th>Annotated genes DE</th>
</tr>
</thead>
<tbody>
<tr>
<td>29d</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>45d</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

### Group effect: AB vs C

- 29d
- 45d

### Age effect: 45d vs 29d

<table>
<thead>
<tr>
<th>Condition</th>
<th>Probes DE</th>
<th>Annotated genes DE</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>2539</td>
<td>1698</td>
</tr>
<tr>
<td>AB</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Hypothesis**: normal maturation of gene expression with age is inhibited by antibiotics through an inhibition of the microbiota implantation.
Results

Transcriptomic study

Diseases and Disorders
Cancer 1181
Infectious Disease 280
Gastrointestinal Disease 640
Hematological Disease 256
Immunological Disease 313

Molecular and Cellular Functions
Cell Growth and Proliferation 558
Cell Death and Survival 543
Gene Expression 362
Cellular Assembly and Organization 255
Cellular Function and Maintenance 420

Physiological System Development and Function
Organismal Survival 386
Hematological System Development and Function 324
Tissue Morphology 315
Hematopoiesis 198
Lymphoid Tissue Structure and Development 170

Top genes up-regulated

<table>
<thead>
<tr>
<th>ID</th>
<th>LogFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>GZMB</td>
<td>2.253</td>
</tr>
<tr>
<td>IRF7</td>
<td>2.146</td>
</tr>
<tr>
<td>CCL5</td>
<td>2.090</td>
</tr>
<tr>
<td>PRF1</td>
<td>1.871</td>
</tr>
<tr>
<td>HSP90B1</td>
<td>1.782</td>
</tr>
</tbody>
</table>

Top genes down-regulated

<table>
<thead>
<tr>
<th>ID</th>
<th>LogFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKNOX2</td>
<td>-2.188</td>
</tr>
<tr>
<td>DCHS1</td>
<td>-2.050</td>
</tr>
<tr>
<td>METTL7A</td>
<td>-1.723</td>
</tr>
<tr>
<td>ARRB1</td>
<td>-1.708</td>
</tr>
<tr>
<td>PTCHD3</td>
<td>-1.648</td>
</tr>
</tbody>
</table>
RESULTS TO COME & CONCLUSION
Results to come

- Promising results with RFF, especially during post-weaning period.
- Waiting for metagenomics results.
Conclusion

Phenotypic study

- With antibiotics: only + 130g in BW at 70d (vs. C) and a lower morbidity rate.

- **BUT ...** no difference between AB and C for parameters of feed intake, weight gain, and feed conversion ratio.

- Moreover, **no difference was found between groups for mortality**
Differential expression quite similar between AB and C for each age in blood.

- **Significantly changes** of the gene expression profile between 29 and 45 days for C group.

- But **no difference** in gene expression was observed for AB group.

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**Leveling of the gene expression in blood by AB**

**Alternative to antibiotics in diet: rapidly fermentable fiber**

**What impacts on cecal microbial ecosystem?**
Thanks to...

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Jean-Marie Bonnemère
Patrick Aymard

GABI unit
Claire Rogel-Gaillard
Jordi Estellé

CRB Gadie Team
Marco Moroldo
Jerôme Lecardonnel
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