健康畜禽 Healthy Livestock

# Dietary Modification of Gut Microbiome and Behavior in Pigs

**JEREMY N. MARCHANT-FORDE**
Research Animal Scientist
USDA-ARS LBRU

**SEVERINE P. PAROIS**
**SUSAN D. EICHER**
**JAY S. JOHNSON**

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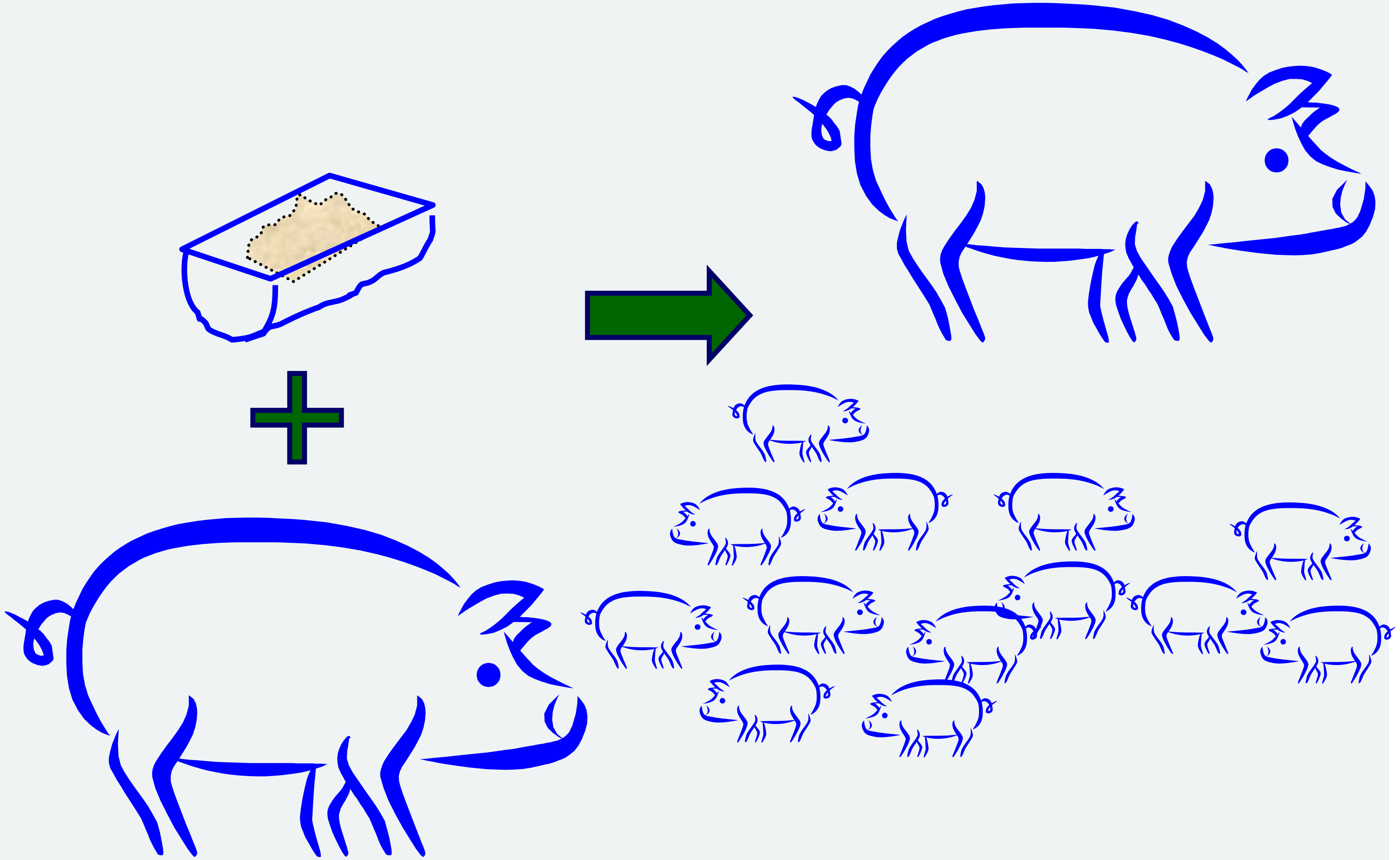
# Introduction

**Put simply,** with pig nutrition management, we aim to.....

.... **Maximize output** for minimal input



# Introduction

**Food** is the most important resource for pigs

Pigs will use aggression to gain or protect food

Thus, feeding system design for pigs in groups will greatly impact the amount of aggression and its consequences

- Delivery system
- Food composition
  - Physical form
  - Flavor
  - **Ingredients**

# Natural vs Commercial Feeding Behavior

| | "Natural" | "Commercial" |
|---|---|---|
| Weaning | Gradual & Late | Abrupt & Early |
| Meals per day | Numerous | Few or one |
| Quality | Variable | Uniform, high |
| Foraging | High proportion of time | Low proportion of time |

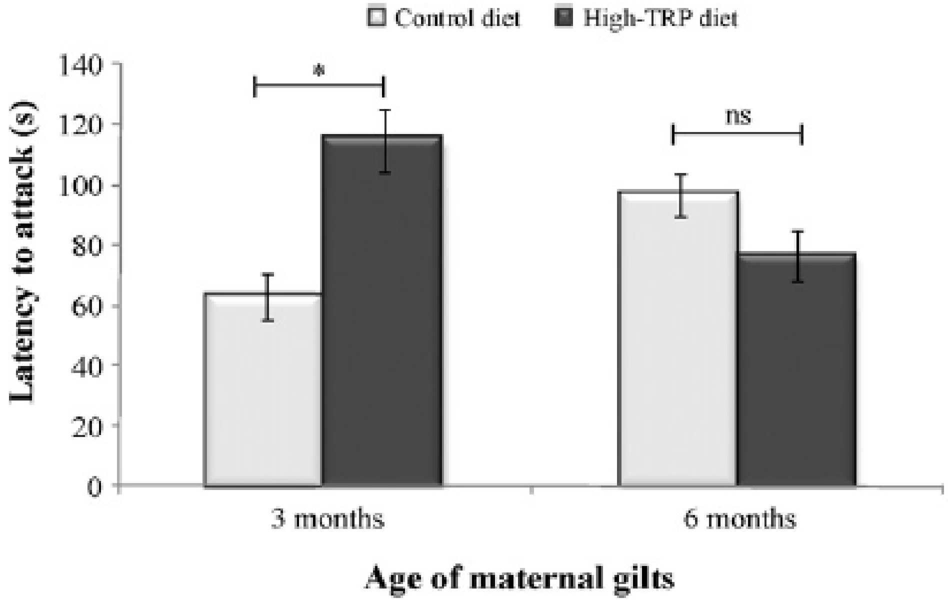
- Microbial populations very different *(Ushida et al 2016)*

# Introduction

- Food composition effects on behavior?
  - “Intentional”
    - e.g. tryptophan to decrease aggression
    - e.g. high fiber to induce satiety
  - “Unintentional”
    - e.g. growth promoters

# Feeding Tryptophan

- Feeding TRP at 250% recommended rate
- Home pen aggression ↓
- Resident-Intruder test aggression ↓
- Serotonin ↑




| | Dietary treatment[a] | |
|---|---|---|
| | Control | High-TRP |
| 3 months | | |
| Number of interactions | 22.8a | 14.8b |
| Bites per interaction | 4.7 | 3.6 |
| Head-knocks per interaction | 2.5 | 2.5 |
| Sum per interaction | 7.2 | 6.2 |

*(Poletto et al., 2010a)*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# Feeding Fibre

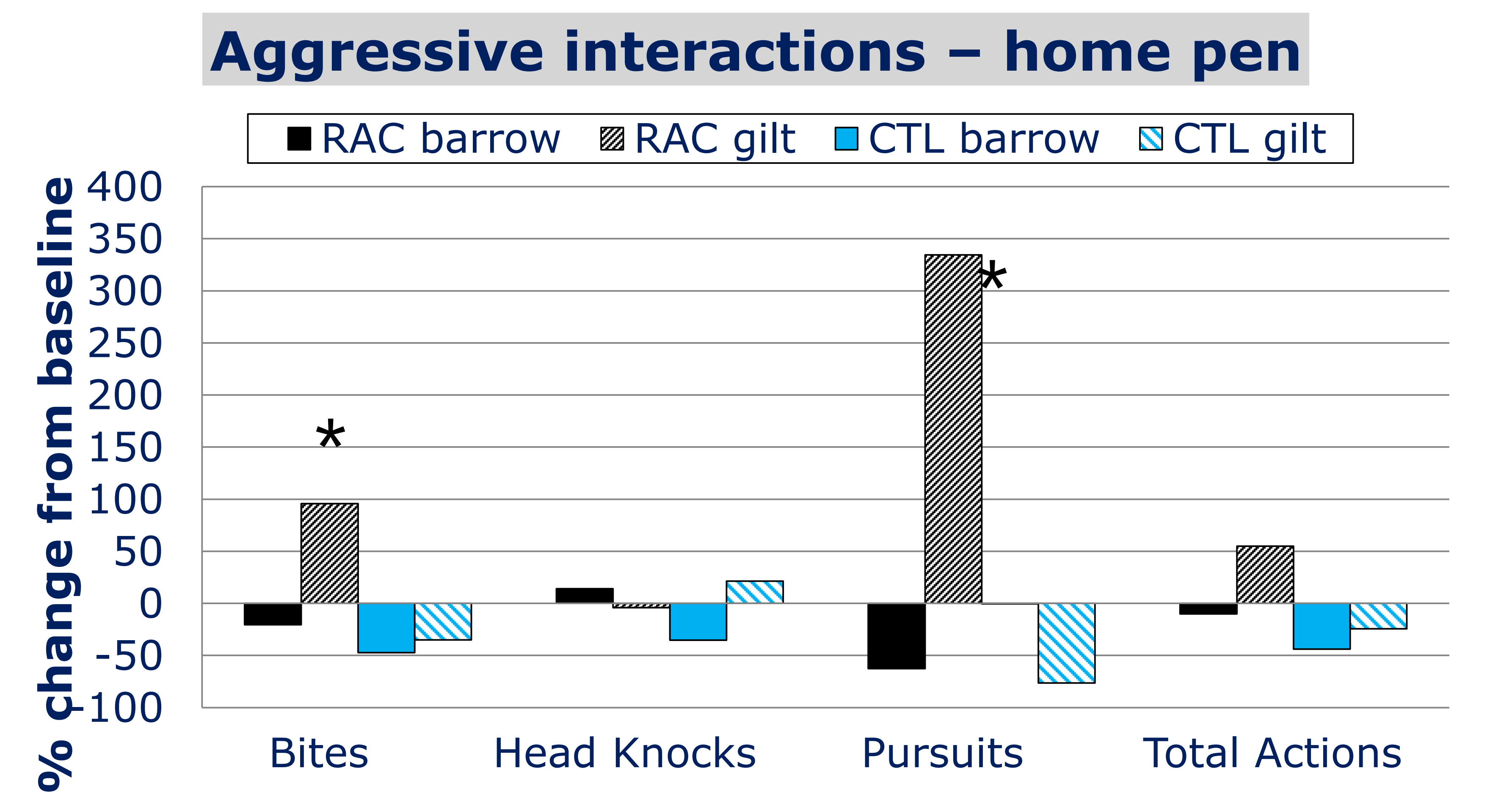
- Bite frequency, fight frequency and fight duration *(Sapkota et al 2016)*

| | Diets | | | | |
|---|---|---|---|---|---|
| | CONTROL[1] | RSTARCH[2] | BEETPULP[3] | SOYHULLS[4] | INCSOY[5] |
| **BF h1** | 236 ± 63[a] | 91 ± 31[b] | 158 ± 41[ab] | 175 ± 22[a] | 111 ± 46[ab] |
| **FF h1** | 19 ± 3[a] | 13 ± 4[b] | 17 ± 3[ab] | 16 ± 3[ab] | 11 ± 5[ab] |
| **FD h1** | 12 ± 5 | 5 ± 2 | 14 ± 6 | 9 ± 3 | 11 ± 5 |

**[1]CONTROL: regular feed with no extra fiber, 2.0 kg/d, 185 g/d NDF**
**[2]RSTARCH: 10.8% resistant starch, 2.0 kg/d, 350 g/d NDF**
**[3]BEETPULP: 27.2 % sugar beet pulp, 2.0 kg/d, 350 g/d NDF**
**[4]SOYHULLS: 19.1% soybean hulls, 2.0 kg/d, 350 g/d NDF**
**[5]INCSOY: 14.05% soybean hulls, 2.2 kg/d, 350 g/d NDF**

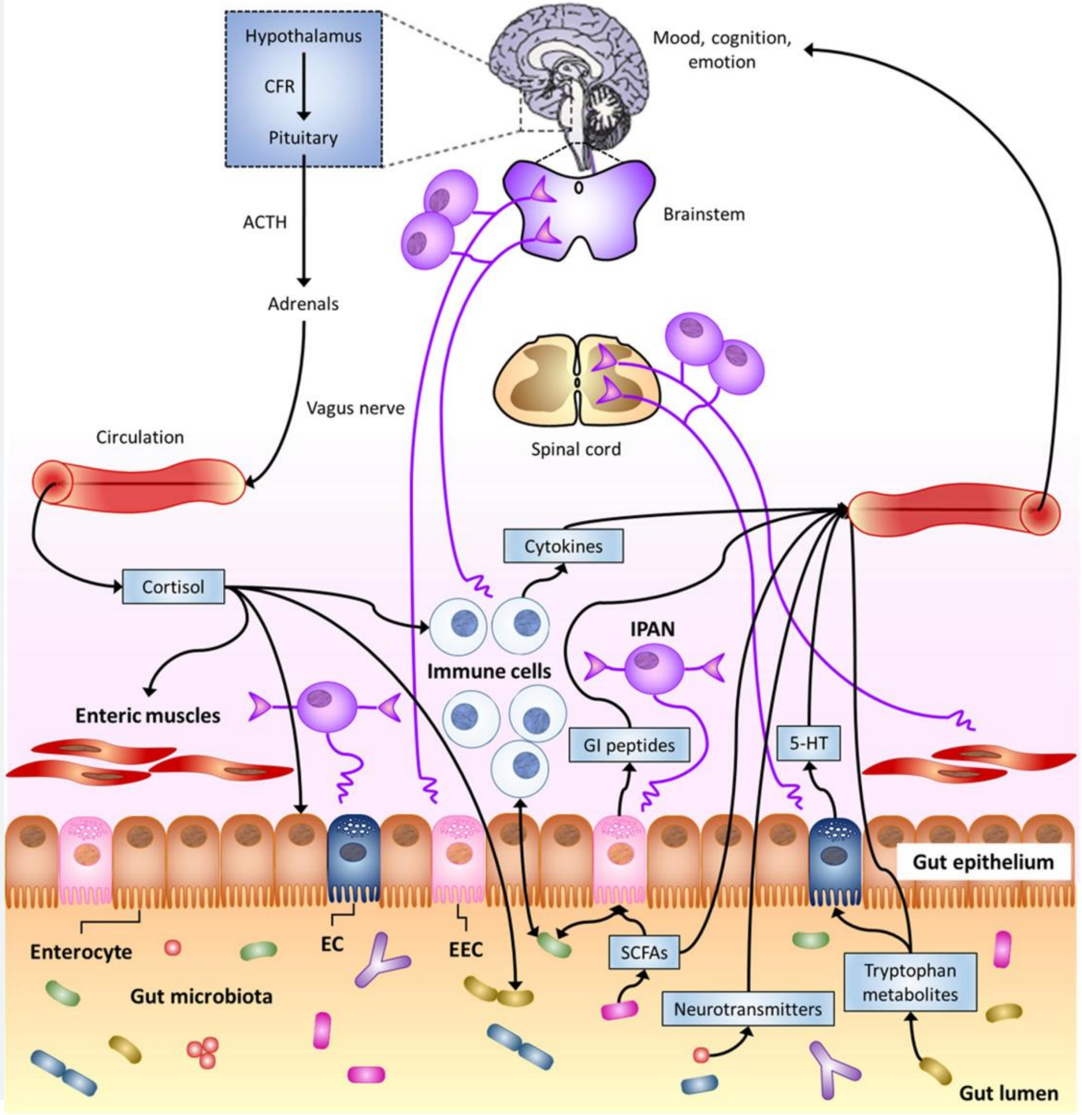
# Ractopamine (Paylean™) effects

*(Poletto et al., 2010a)*



# Gut-Brain Axis

- Gut microbiota communicates with the CNS — possibly through neural, endocrine and immune pathways — and thereby influences brain function and behavior
- Suggests a role for the gut microbiota in the regulation of anxiety, mood, cognition and pain

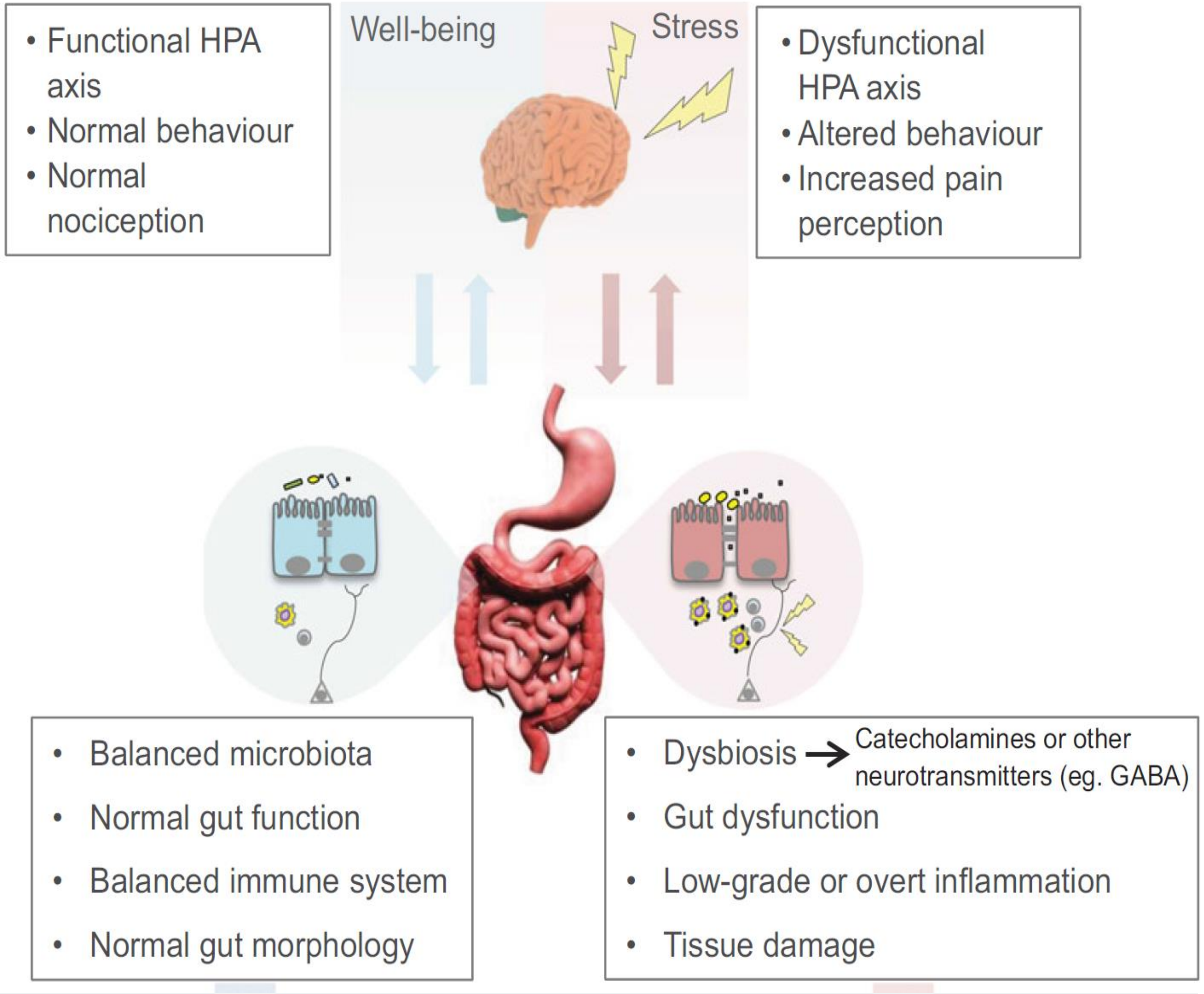



*(Mazzoli & Pessione 2016 from Cryan & Dinan, 2012)*

# Gut-Brain Axis

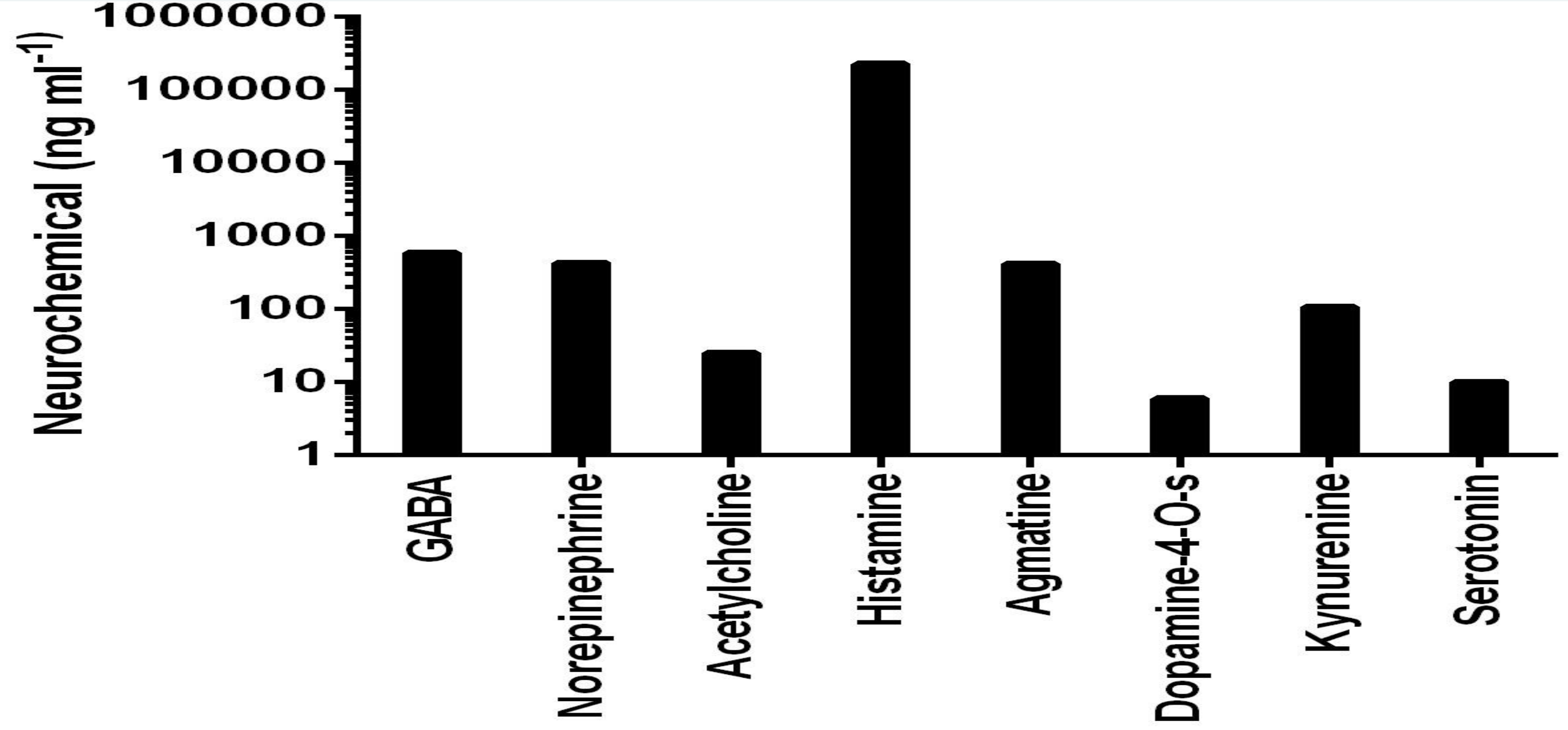
- 2-way street!
- Brain can influence microbiota
- Microbiota can influence brain

*(De Palma et al., 2014)*



# Biogenic Amines and Bacteria

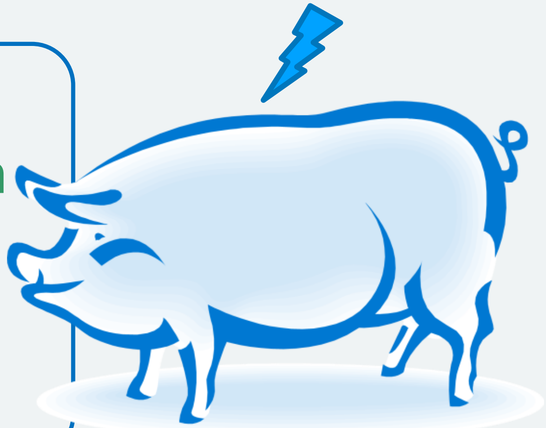
- Lactobacillus neurochemical production



# Context

- Pigs are subject to stress at various timepoints of production

*Stressors*



**Weaning**
- Sow separation
- Mixing
- Diet change

**Transport**
- Dehydration
- Feed restriction
- Temperature extremes

Emotional distress | ↑ Pathogen exposure | ↓ Intestinal health | ↓ Energy intake

➡ **Performance + Welfare issues**

(Chiba, 2010; Smith et. al., 2010; Campbell et al., 2013)

# Context

Societal demand for a **change in farm animal practices**

- Reduction of **antibiotics use**
- Improvement of farm animal **welfare**

Feed additives are promising products for that purpose

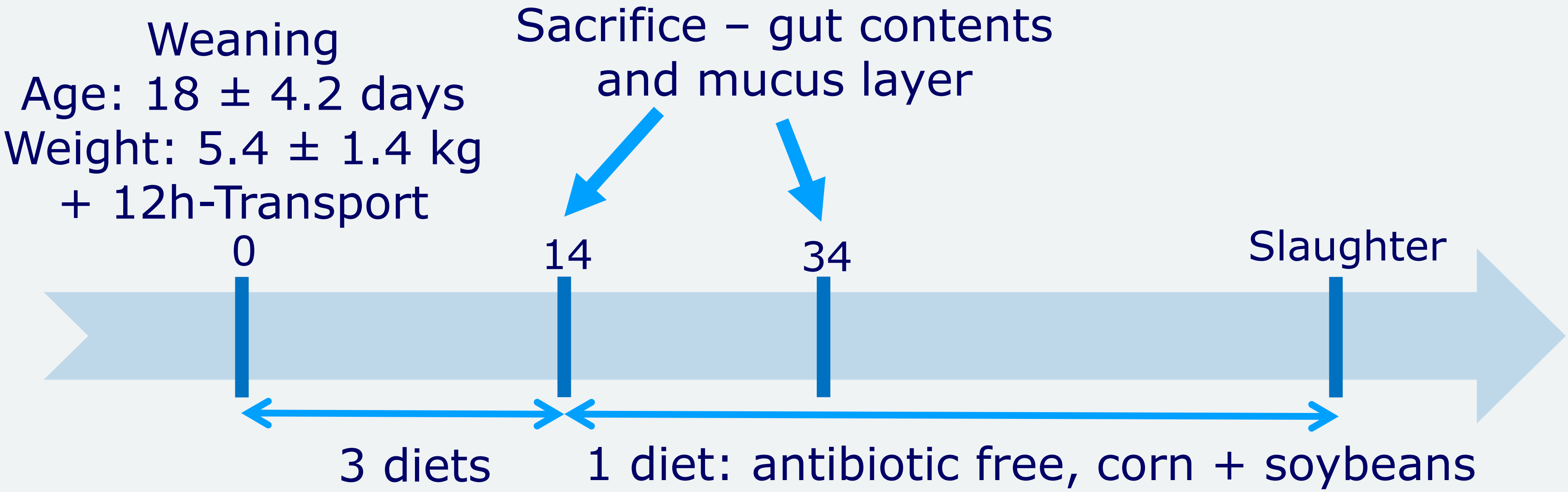
- Reduction of **stress**
- Improvement of **Health** + **Reproductive performance**
- Better **recovery** from adverse events *(farrowing, weaning…)*

Antibiotic alternatives:

- Bacteriophages, **Probiotics**, **Prebiotics**, Organic acids, Plant extracts, Essential oils, Lysozymes, **Amino acids**

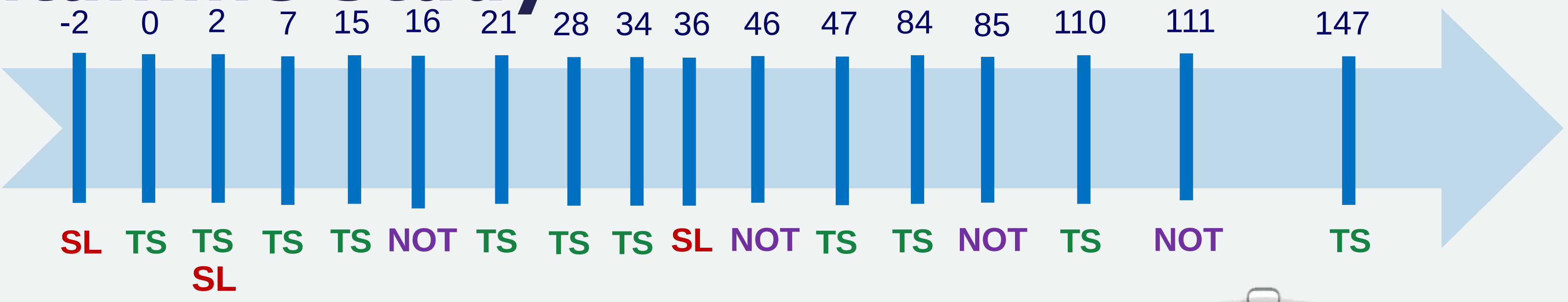
# L-Glutamine Study

**240** (Yorkshire × Landrace × Duroc) *From 32 litters, Groups of 8*




1. **A** – Antibiotic diet: Chlortetracycline (0.40g/kg) + Tiamulin (0.035g/kg)
2. **NA** – Control diet: without any antibiotic or feed supplement
3. **GLN** – L-glutamine diet: 0.20% L-glutamine

# L-Glutamine Study




**TS** – Tear staining
(indicator of stress)






**SL** – Skin lesions
(indicator of aggression)


**NOT** – Novel object test in home pen

*Avoidance*

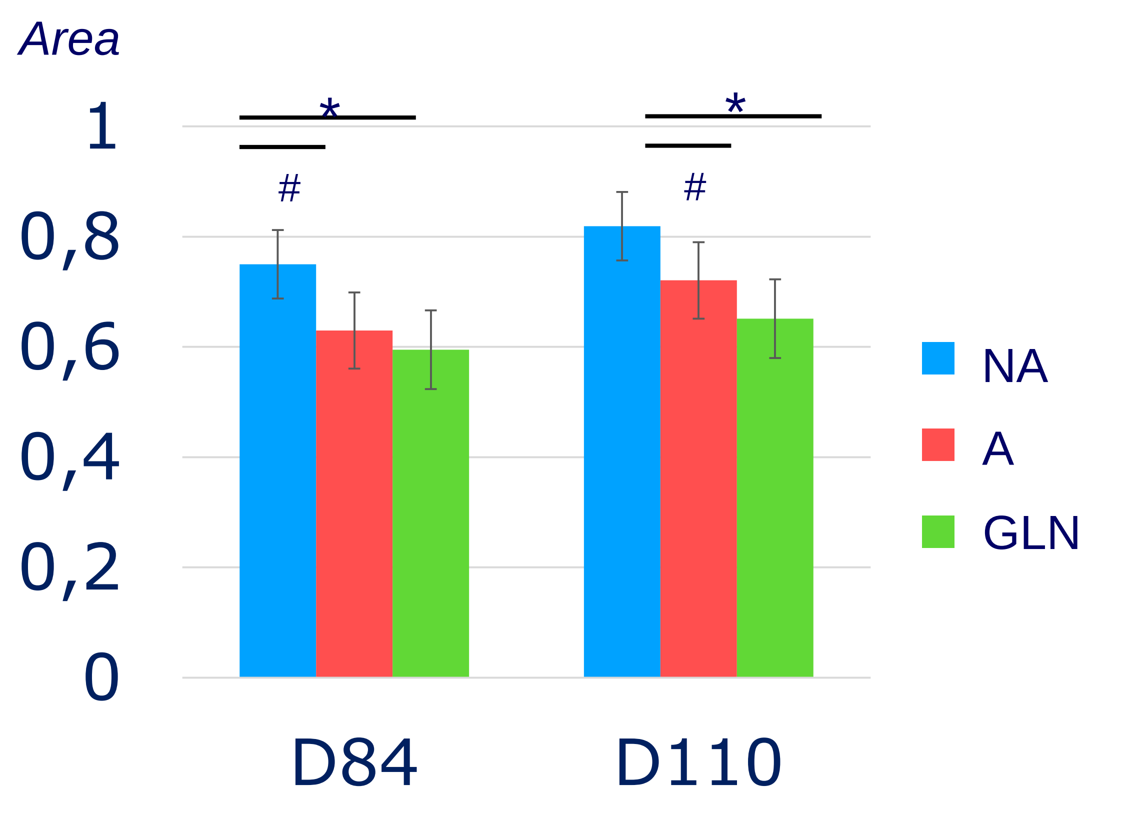
*Showing interest*

*Interacting*

# L-Glutamine Results

- Larger stain areas for **NA** pigs = Long-term effects of a short diet treatment
- **NA** pigs more stressed
- **GLN** pigs similar to **A** pigs

## Tear Stains

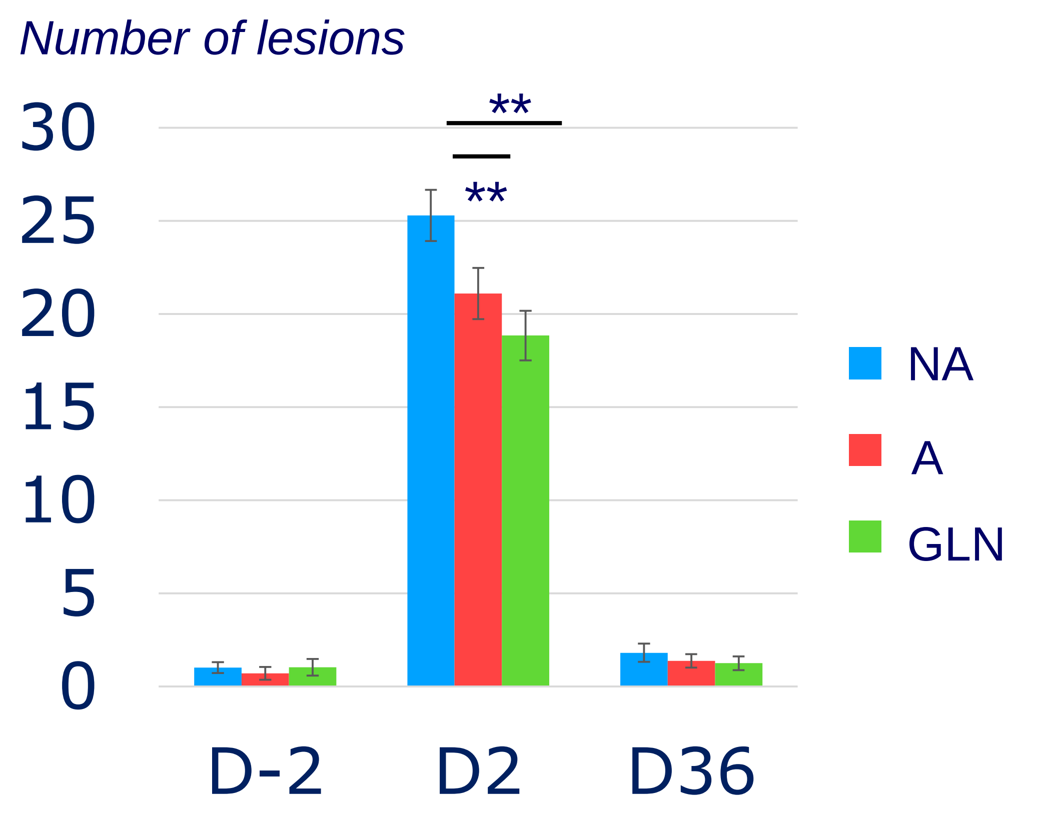



# p<0.1   * p<0.05

# L-Glutamine Results

- Effects only on Day 2 – the establishment of the hierarchy post-mixing
- **NA** pigs had more lesions than **GLN** and **A** pigs
- **GLN** pigs similar to **A** pigs

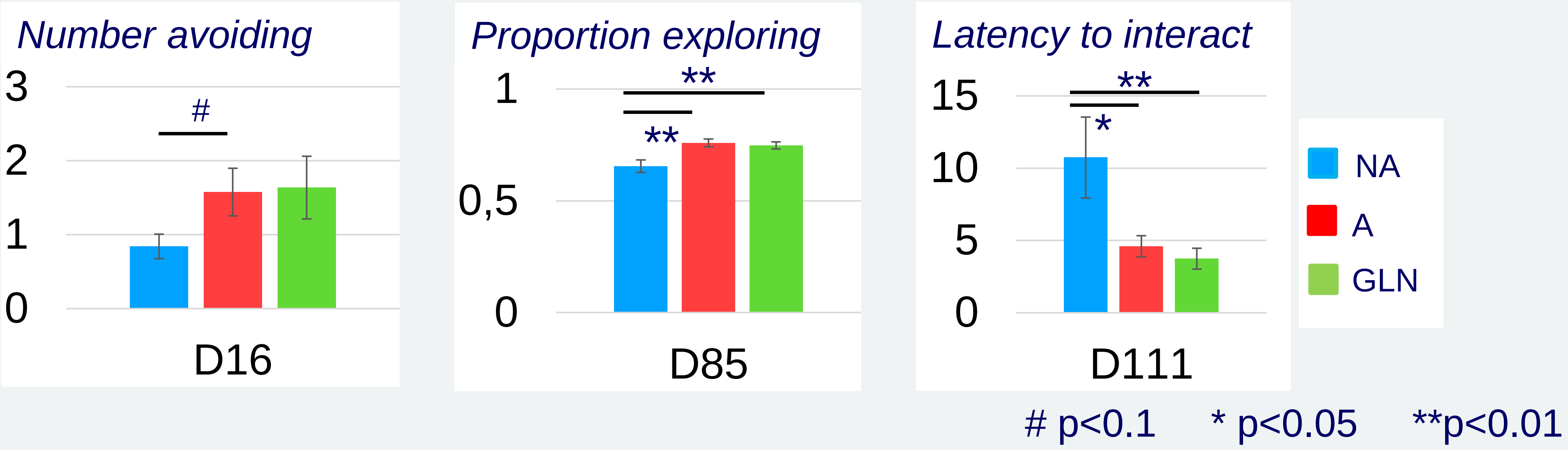
## Skin Lesions




** p<0.01

# L-Glutamine Results

## Novel Object Tests




- Short- and long-term effects of a short term diet treatment
- NA pigs less interested by object – less avoiding, but less exploring and slower to interact

# L-Glutamine Results

- Three main phyla were found across all samples:
- *Firmicutes* represented 77.5% of the total sequenced DNA,
  - *Clostridia* (57.6%)
  - *Bacilli* (28.6%)
  - *Negativicutes* (9.4%)
  - *Erysipelotrichia* (3.7%)
- *Bacteroidetes* 13.5%
  - *Bacteroidia* (95.4%)
  - *Bacteroidetes unclassified* (4.6%)
- *Proteobacteria* 4.9%
  - *Gammaproteobacteria* (49.7%)
  - *Proteobacteria unclassified* (23.0%)
  - *Epsilonproteobacteria* (19.7%)
  - *Betaproteobacteria* (3.6%)
  - *Deltaproteobacteria* (3.4%)

# L-Glutamine Results

- Differences between dietary treatments were demonstrated at d14 but had disappeared at d34.
- GLN pigs had higher richness, evenness and diversity than A and NA pigs

| Traits | Treatment | | | |
|---|---|---|---|---|
| | A | GLN | NA | *P* |
| Chao1 estimator | 219.0±6.1[a] | 254.3±6.1[b] | 224.3±5.9[a] | <0.001 |
| ACE estimator | 257.5±7.1[a] | 303.1±7.0[b] | 271.1±6.8[a] | <0.001 |
| Shannon index | 3.17±0.05[a] | 3.34±0.05[b] | 3.18±0.04[a] | 0.013 |
| InvSimpson index | 15.2±0.7[a] | 18.6±0.7[b] | 15.1±0.7[a] | <0.001 |
| sobs | 141.6±4.2[a] | 162.7±4.1[b] | 142.4±3.3[a] | <0.001 |

- Multiple correlations between lesions, tear staining, NOT behavior and specific bacterial populations, but only up to D28

# L-Glutamine Results

- Other differences between dietary treatments:

(*see Duttlinger et al., 2019 doi: 10.1093/jas/skz098*)

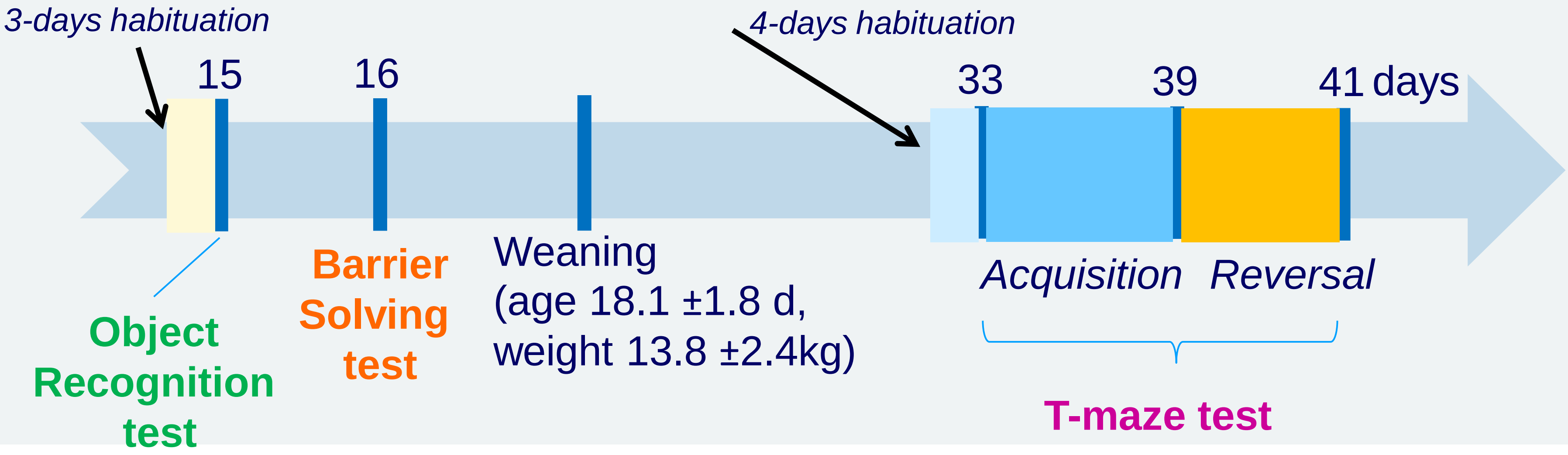
| Parameters | Treatment | | | |
|---|---|---|---|---|
| | A | GLN | NA | *P* |
| TNF-2$^{\alpha}$ pg/mL D13 | $36.73^{a}$ | $40.92^{a}$ | $63.19^{b}$ | 0.02 |
| ADG g D0-14 | $224^{a}$ | $210^{a}$ | $189^{b}$ | <0.01 |
| ADFI g D0-14 | $277^{a}$ | $272^{a}$ | $253^{b}$ | 0.04 |
| BW kg D14 | $8.65^{a}$ | $8.50^{a}$ | $8.19^{b}$ | <0.01 |
| Aggression % D2-12 | $1.74^{x}$ | $1.28^{y}$ | $1.41^{xy}$ | <0.1 |

# Synbiotic Study


(Landrace × York)

From **1 to 28 days of age**, individual feed supplementation by oral dosing:

1. **SYN** - Synbiotic supplement: a probiotic (*Lactobacillus)* + a prebiotic (fructo-oligosaccharide) + *Saccharomyces cerevisiae* cell wall
2. **CTL -** Control supplement: chocolate milk



# Synbiotic Study

## Episodic-like memory test

**OR test** = remembering an object already explored 50 min before (24h prior exposure to a different object on farrowing crates for acclimation to objects)

# Synbiotic Study

## Working memory test

**BS test** = finding a route through 2 barriers to join 2 companion pigs over 5 successive trials

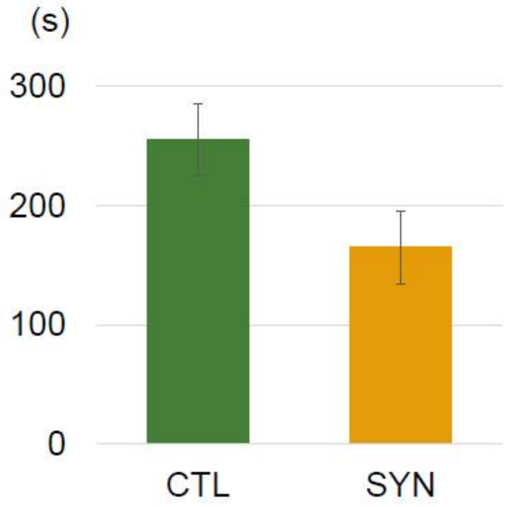
# Synbiotic Study

## Spatial, working and reference memories test

**T-maze test** = finding a food reward
2 periods: an acquisition stage (similar arm rewarded)
+ a reversal stage (switched arm)

# Synbiotic Results

*Fig1. Latency to interact with the novel object*

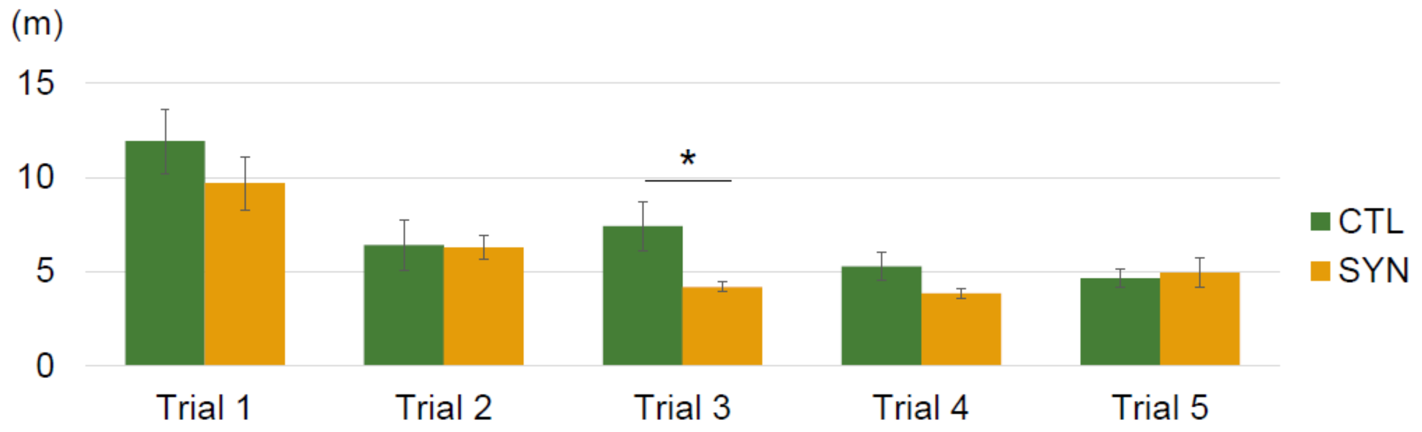



Both treatment groups explored the reference objects and environment the same way (distance travelled, frequency and duration of interaction; $p>0.1$).

**SYN piglets interacted quicker with the novel object** than the CTL piglets (165 ±116 vs 255 ±120s; $p<0.05$).

# Synbiotic Results

*Fig2. Distance travelled to reach the companion pigs*



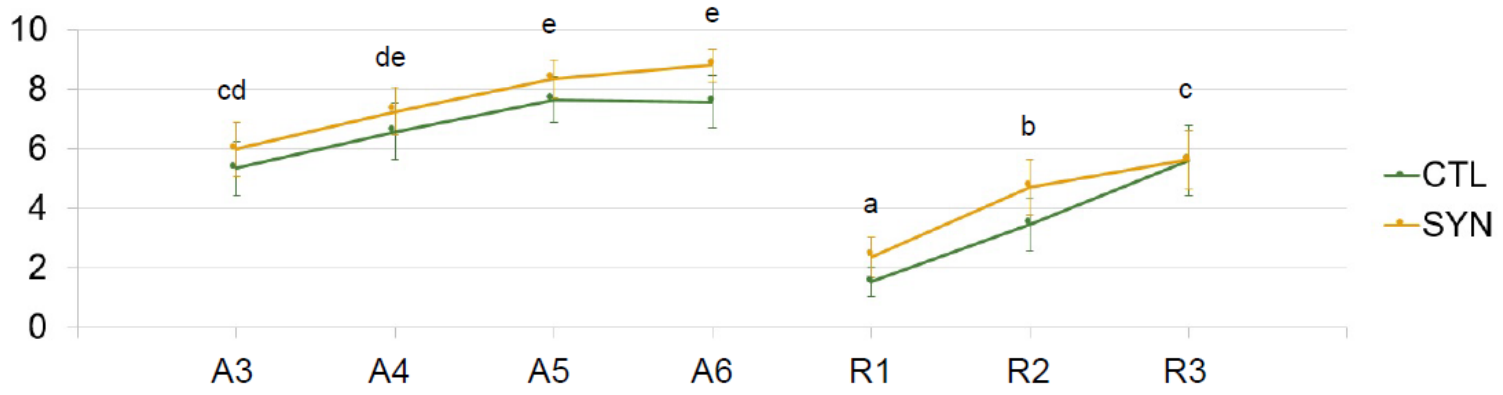

No differences regarding the times needed to cross each barrier and to finish the test (*p*>0.1).

Performances in **trial 1 were lowest for all traits** (*p*<0.001): progression over time. - WORKING MEMORY

**SYN piglets had shorter distances** to finish the test in **trial 3** (4.2 ±1.0 vs 7.4 ±5.2m; *p*<0.05). - WORKING MEMORY

# Synbiotic Results

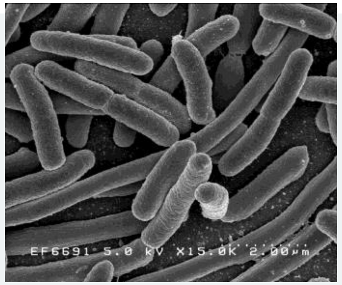
*Fig3. Number of correct choices*




Number of correct choices and time to succeed were similar (*p*>0.1), except on **day 3** of the acquisition stage where **SYN piglets were quicker** than CTL piglets (13.0 ±6.2 vs 25.8 ±18.3s; *p*<0.05). - SPATIAL MEMORY

During the reversal stage, **SYN piglets tried the new rewarded arm earlier** than CTL piglets (10.3 ±10.9 vs 19.8 ±15.4 trials; *p*<0.05). - REFERENCE MEMORY

# Synbiotic Results



- Again, three main phyla were found across all samples:

- *Firmicutes* represented 70.9% of the total sequenced DNA,
    - *Clostridia* (60.7%)
    - *Bacilli* (20.6%)
    - *Negativicutes* (12.0%)

- *Bacteroidetes* 19.2%
    - *Bacteroidia* (88.2%)
    - *Bacteroidetes unclassified* (11.8%)
- *Proteobacteria* 3.2%
    - *Gammaproteobacteria* (44.0%)
    - *Deltaproteobacteria* (30.4%)
    - *Epsilonproteobacteria* (17.0%)
    - *Betaproteobacteria* (5.5%)

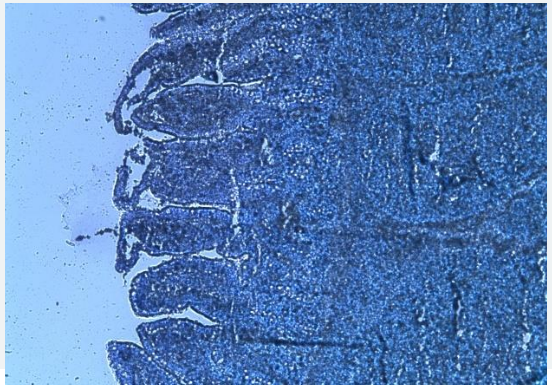
# Synbiotic Results

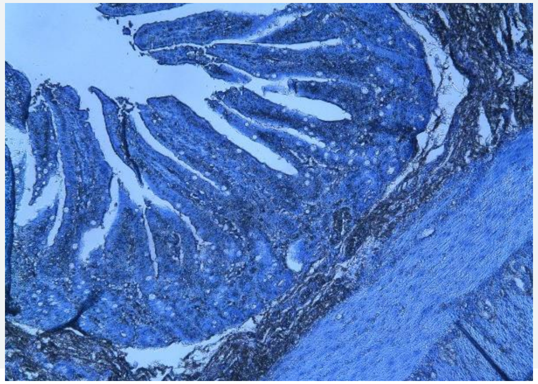
- No differences in microbiota between treatments at d15
- Tendency at d33 (P<0.066)
- Different at d39 (P<0.047)
- Suggests we may have long-term impact on microbiota if we dose early

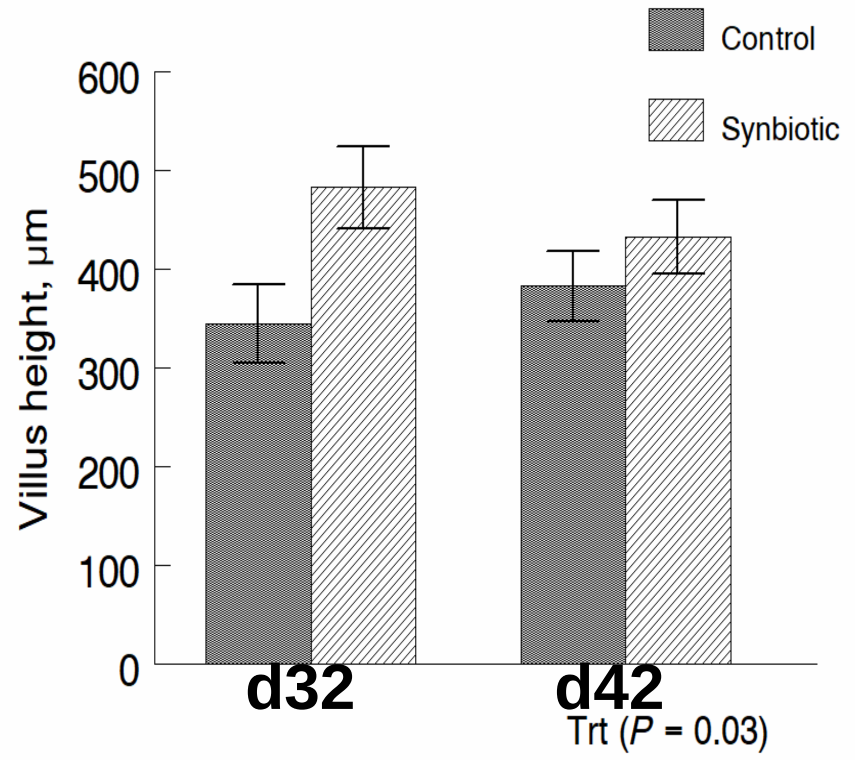
| | Day | CTL | CTL | CTL | PRO | PRO | PRO |
|---|---|---|---|---|---|---|---|
| | | 15 | 33 | 39 | 15 | 33 | 39 |
| **CTL** | 15 | | <0.001 | <0.001 | NS | <0.001 | <0.001 |
| **CTL** | 33 | | | 0.012 | <0.001 | 0.066 | 0.003 |
| **CTL** | 39 | | | | <0.001 | 0.004 | 0.047 |
| **PRO** | 15 | | | | | <0.001 | <0.001 |
| **PRO** | 33 | | | | | | 0.025 |
| **PRO** | 39 | | | | | | |

# Synbiotic Results

- Post-weaning villus height greater in SYN piglets
- Counts of jejunal tissue E. coli were greater for CTL pigs on day 32
- Lactic acid bacteria (LAB) that includes Lactobacillus were greater for SYN pigs on day 32







# Conclusions

- Short-term feeding strategy (2 or 4 weeks) can have both short- and long-term effects
- **Study 1:** L-glutamine (**GLN**) appeared to confer similar benefits to, and thus could be a viable alternative to dietary antibiotics
- **Study 2:** The synbiotic supplement may **confer memory advantages in the 3 cognitive tasks**, regardless of the nature of the reward and the memory request.
- Beneficial effects occurred both **before** and **after weaning**

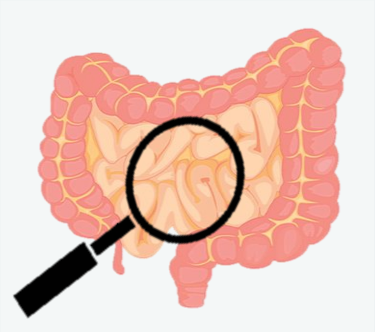
# Untapped Potential

*Gut-Brain axis*

Interactions

- ✓ **Behavior**
- ✓ **Memory**
- ✓ **Coping with stress**

✓ **Diet**



Microbiota

"the relationship between diet and the microbiota-gut-brain axis is ripe for exploitation to develop therapeutic strategies for treating stress-related disorders"

Foster et al. (2017) Stress & the gut-brain axis: Regulation by the microbiome. *Neurobiology of Stress*, **7:**124-136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