# EFFICIENT & ECOLOGICALLY-FRIENDLY PIG AND POULTRY PRODUCTION.

ECOFCE

## A WHOLE-SYSTEMS APPROACH TO OPTIMISING FEED EFFICIENCY AND REDUCING THE ECOLOGICAL FOOTPRINT OF MONOGASTRICS.

### BASIC DATA

**Funding:**
EU-FP7
(€ 6 million)

**Start date:**
1 February 2013

**Duration:**
48 months
(2013 to 2017)

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 311794.

# TRIAL Nº B-443 (IRTA)
# ECO-FCE PROJECT. TRIAL Nº1
# WP2: IDENTIFICATION AND OPTIMISATION OF FEED STRATEGIES

IRTA-CReSA

Results of Intestinal Microbiota (deep sequencing), Intestinal Mucosa Histology, Intestinal Mucosa Immunological Parameters

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 311794.

# OBJECTIVES

- The main objective was to investigate nutritional conditioning as a feeding regime strategy to improve the lifetime performance and nutrient utilisation of broiler chickens.
- In the present trial, the target nutrients were phosphorus and calcium, and the specific objective was to analyse differences between good and poor FCR animals.
- The criteria to evaluate the effects of P/Ca nutritional conditioning were:

  Productive parameters (gain and efficiency), gut morphology and immunology, and intestinal microbiota composition in birds allocated individually from 14-22 days and from 22-30 days.

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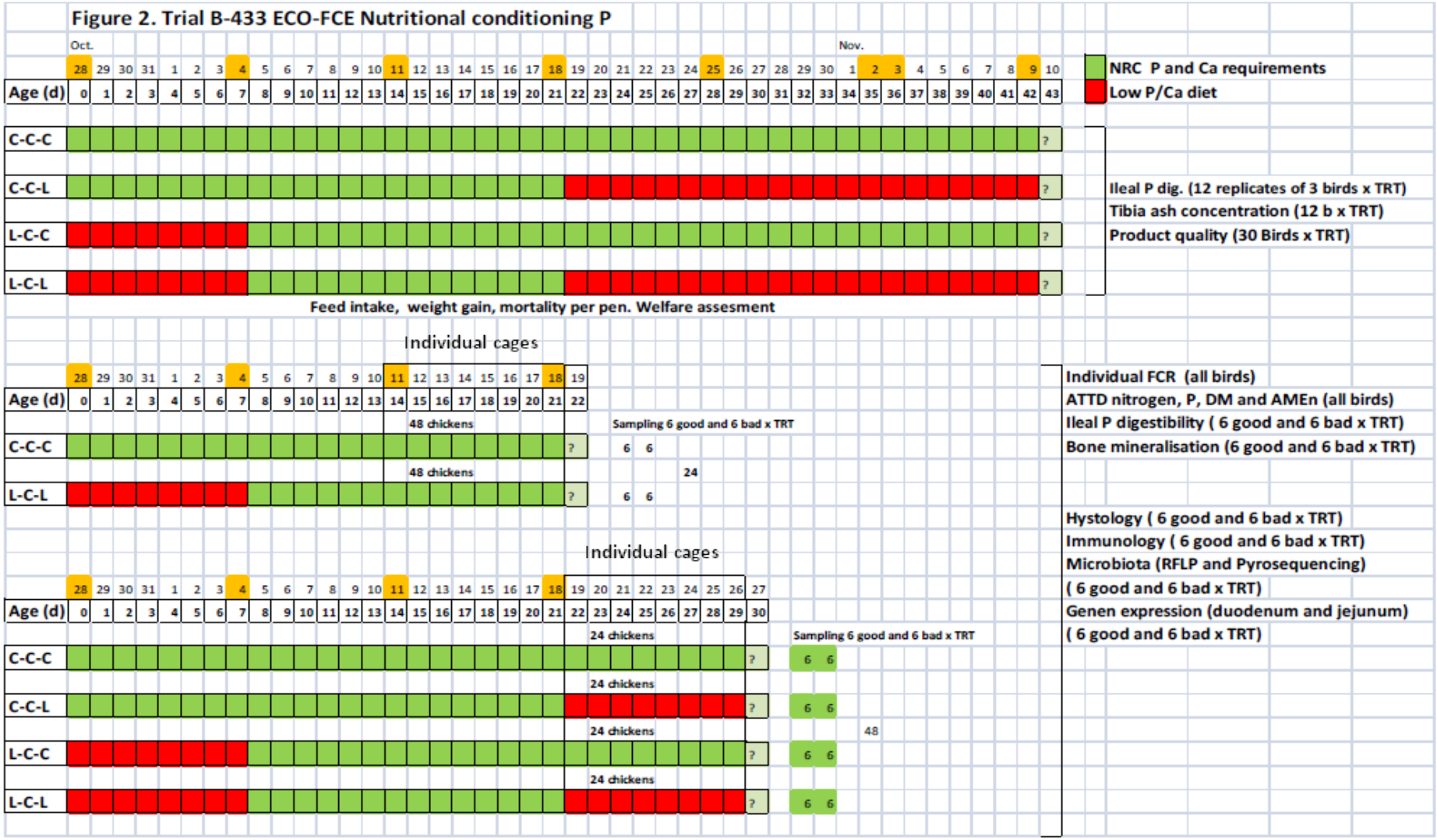
# Trial scheduler

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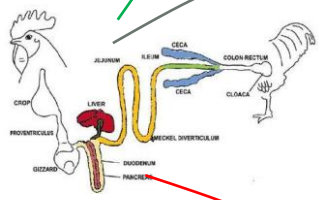
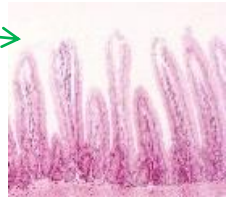
# Sampling and analytical methods



Formol

Formol

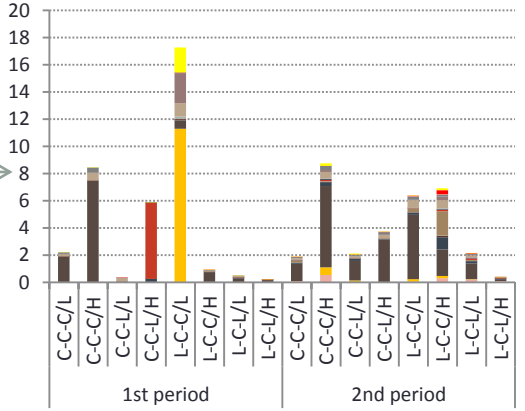


HE stain (VH, CD, VH:CD ratio)

Etanol

Etanol

Ion Torrent 316™ Chip Kit



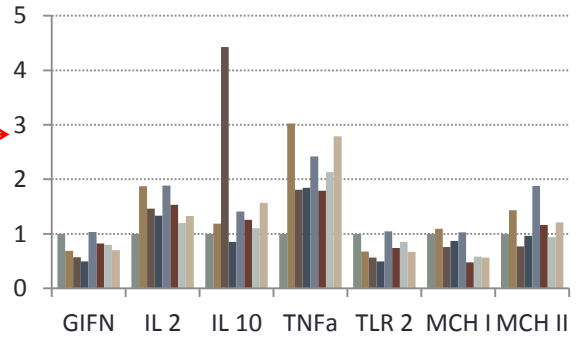
RNA-Later

22d

RNA-Later

30d

Q-PCR and $2^{-\Delta\Delta C_T}$

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# First Period Histology

| Factor | | Villi height (µm) | | Crypt depth (µm) | | VH:CD ratio | |
|---|---|---|---|---|---|---|---|
| **Feed conversion rate** | | **25% PFC** | **25% GFC** | **25% PFC** | **25% GFC** | **25% PFC** | **25% GFC** |
| | **Average** | 627.20 | 594.80 | 166.93 | 158.27 | 4.02 | 3.88 |
| | **SE** | 11.26 | 12.22 | 3.98 | 3.37 | 0.12 | 0.09 |
| | ***P*** | **0.052** | | **0.098** | | 0.35 | |
| **Starter diet** | | **Control diet** | **Low P/Ca diet** | **Control diet** | **Low P/Ca diet** | **Control diet** | **Low P/Ca diet** |
| | **Average** | 618.87 | 603.13 | 162.40 | 162.80 | 4.05 | 3.84 |
| | **SE** | 10.15 | 13.28 | 4.01 | 3.38 | 0.11 | 0.10 |
| | ***P*** | 0.35 | | 0.94 | | 0.16 | |

Anova(model.glm,type='II',test.statistic='Wald') Analysis of Deviance Table (Type II tests)
PFC=Poor Feed conversion rate
GFC=Good Feed conversion rate
SE=Standard error

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# Second Period Histology

| Factor | | Villi height (µm) | | Crypt depth (µm) | | VH:CD ratio | |
|---|---|---|---|---|---|---|---|
| **Feed conversion rate** | | **25% PFC** | **25% GFC** | **25% PFC** | **25% GFC** | **25% PFC** | **25% GFC** |
| | **Average** | 628.73 | 716.57 | 146.03 | 115.93 | 4.67 | 6.51 |
| | **SE** | 6.70 | 6.31 | 2.68 | 1.93 | 0.10 | 0.10 |
| | ***P*** | **<0.0001** | | **<0.0001** | | **<0.0001** | |
| **Starter diet** | | **Control diet** | **Low P/Ca diet** | **Control diet** | **Low P/Ca diet** | **Control diet** | **Low P/Ca diet** |
| | **Average** | 664.67 | 680.63 | 131.77 | 130.20 | 5.54 | 5.64 |
| | **SE** | 7.55 | 6.58 | 2.59 | 2.47 | 0.12 | 0.11 |
| | ***P*** | 0.11 | | 0.66 | | 0.54 | |
| **Finisher diet** | | **Control diet** | **Low P/Ca diet** | **Control diet** | **Low P/Ca diet** | **Control diet** | **Low P/Ca diet** |
| | **Average** | 688.37 | 656.93 | 139.97 | 122.00 | 5.32 | 5.86 |
| | **SE** | 7.51 | 6.51 | 2.51 | 2.41 | 0.11 | 0.12 |
| | ***P*** | **0.0017** | | **<0.0001** | | **<0.001** | |

Anova(model.glm,type='II',test.statistic='Wald') Analysis of Deviance Table (Type II tests)
PFC=Poor Feed conversion rate
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SE=Standard error

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# Q-PCR Immunological Parameters of Ileal Mucosa

**First Period**

| | GIFN | IL 2 | IL 10 | TNFa | TLR 2 | MCH I | MCH II |
|---|---|---|---|---|---|---|---|
| C-C/P | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| C-C/G | 1.174 | 0.599 | 0.296 | 0.473 | 1.127 | 2.079 | 0.526 |
| L-C/P | 1.173 | 0.732 | 0.261 | 0.818 | 1.158 | 2.636 | 0.876 |
| L-C/G | 0.719 | 0.672 | 1.356 | 0.578 | 0.943 | 2.574 | 0.676 |
| Feed conversion rate[1] | *P*=0.908 | *P*=0.356 | *P*=0.603 | *P*=0.204 | *P*=0.817 | *P*=0.644 | ***P*=0.094** |
| Starter diet[1] | *P*=0.773 | *P*=0.386 | *P*=0.862 | *P*=0.773 | *P*=0.954 | *P*=0.908 | *P*=0.908 |

**Second Period**

| | GIFN | IL 2 | IL 10 | TNFa | TLR 2 | MCH I | MCH II |
|---|---|---|---|---|---|---|---|
| C-C-C/P | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| C-C-C/G | 0.685 | 1.873 | 1.184 | 3.022 | 0.678 | 1.095 | 1.434 |
| C-C-L/P | 0.571 | 1.459 | 4.426 | 1.811 | 0.565 | 0.760 | 0.771 |
| C-C-L/G | 0.492 | 1.332 | 0.851 | 1.845 | 0.491 | 0.869 | 0.963 |
| L-C-C/P | 1.034 | 1.884 | 1.409 | 2.417 | 1.044 | 1.029 | 1.882 |
| L-C-C/G | 0.825 | 1.533 | 1.254 | 1.790 | 0.741 | 0.475 | 1.163 |
| L-C-L/P | 0.799 | 1.197 | 1.104 | 2.128 | 0.852 | 0.584 | 0.939 |
| L-C-L/G | 0.699 | 1.325 | 1.566 | 2.787 | 0.668 | 0.562 | 1.211 |
| Feed conversion rate[1] | ***P*=0.070** | *P*=0.257 | *P*=0.718 | *P*=0.174 | ***P*=0.080** | *P*=0.885 | *P*=0.509 |
| Starter diet[1] | *P*=0.409 | *P*=0.934 | *P*=0.766 | *P*=0.257 | *P*=0.433 | *P*=0.208 | *P*=0.446 |
| Finisher diet[1] | *P*=0.143 | *P*=0.564 | *P*=0.566 | *P*=0.550 | *P*=0.224 | *P*=0.470 | *P*=0.343 |

[1]Kruskal-Wallis rank sum test

C= Normal P/Ca diet; L= Low P/Ca diet; G= Good FCR; P= Poor FCR

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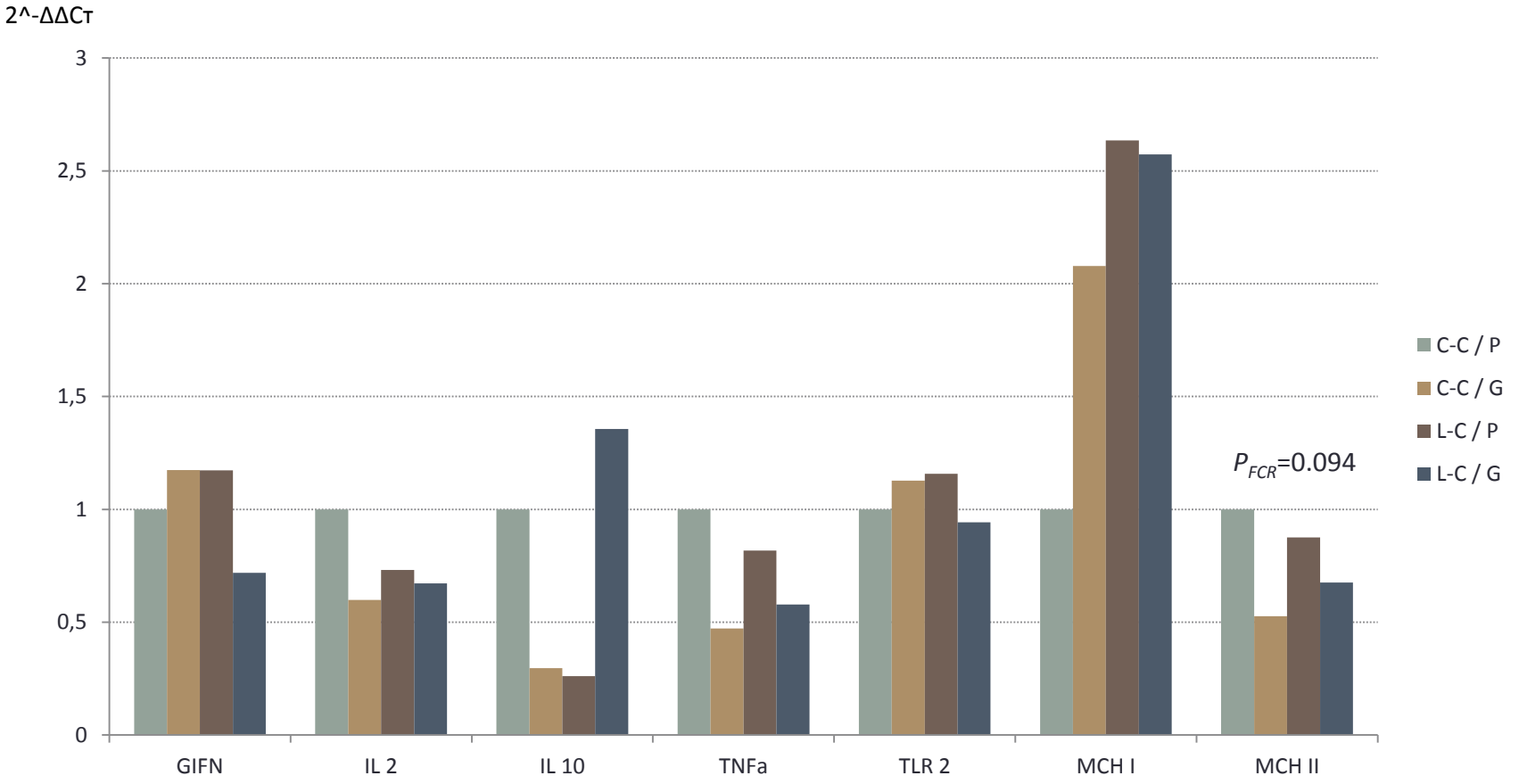
# First Period
# Q-PCR Immunological Parameters of Ileal Mucosa

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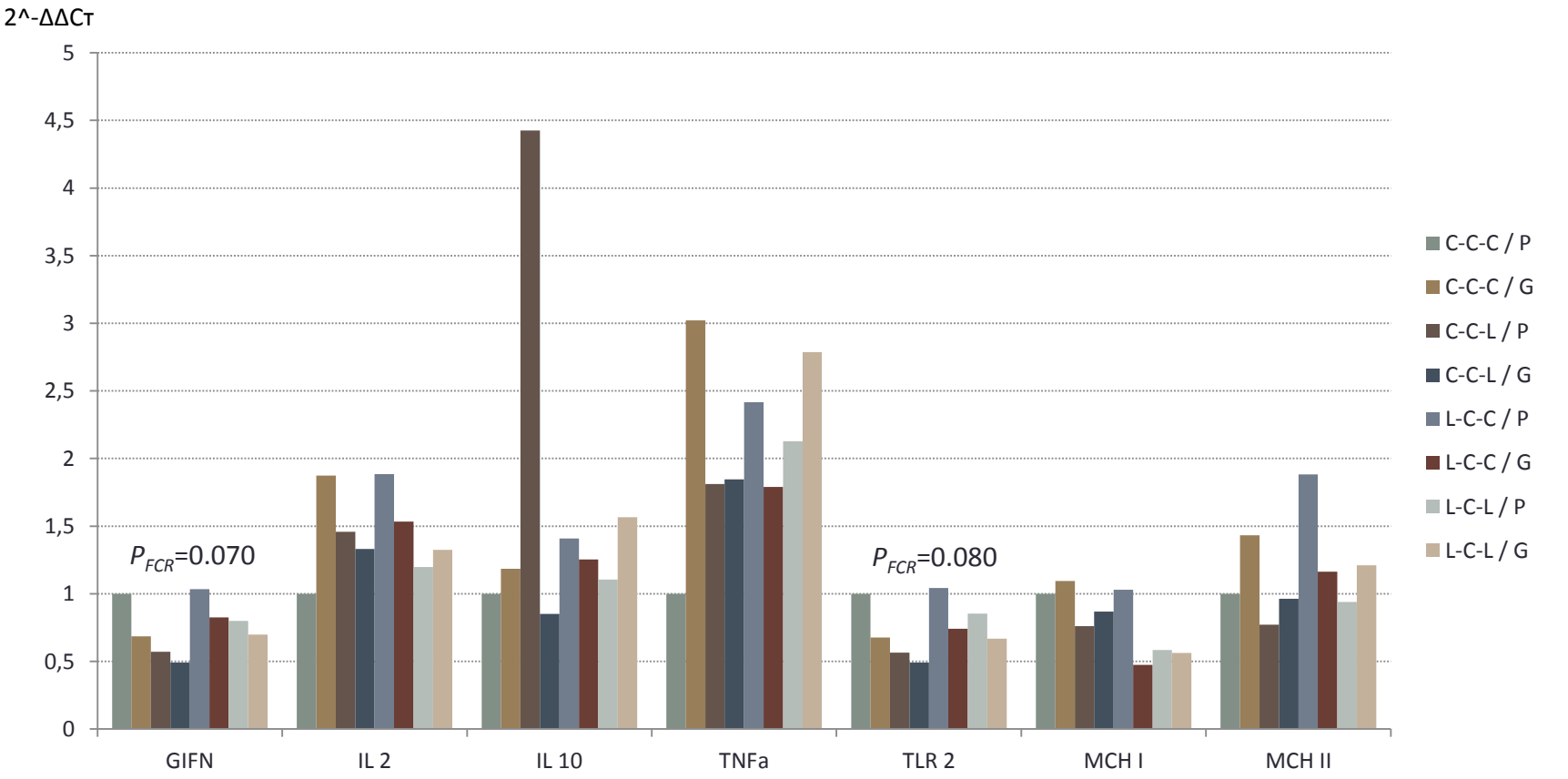
# Second Period
# Q-PCR Immunological Parameters of Ileal Mucosa

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# Summary of Ion-Torrent analysis of Ileal Microbiota

| | First Period | | | |
|---|---|---|---|---|
| | C-C/G | C-C/P | L-C/G | L-C/P |
| **Number of sequences** | 345221 | 353196 | 382219 | 311926 |
| **% Lactobacillus** | 91.74 | 86.38 | 83.36 | 93.99 |
| **% Other bacteria** | 8.26 | 13.62 | 16.64 | 6.01 |
| **Biodiversity** | 78 | 60 | 89 | 57 |

C= Normal P/Ca diet; L= Low P/Ca diet; G= Good FCR; P= Poor FCR

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# Summary of Ion-Torrent analysis of Ileal Microbiota

| | Second Period | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | C-C-C/G | C-C-C/P | C-C-L/G | C-C-L/P | L-C-C/G | L-C-C/P | L-C-L/G | L-C-L/P |
| **Number of sequences** | 125949 | 181309 | 173262 | 201476 | 197613 | 200657 | 166206 | 155863 |
| **% Lactobacillus** | 91.19 | 83.62 | 91.07 | 90.61 | 86.99 | 84.98 | 90.82 | 93.75 |
| **% Other bacteria** | 8.81 | 16.38 | 8.93 | 9.39 | 13.01 | 15.02 | 9.18 | 6.25 |
| **Biodiversity** | 84 | 93 | 71 | 50 | 86 | 135 | 101 | 68 |

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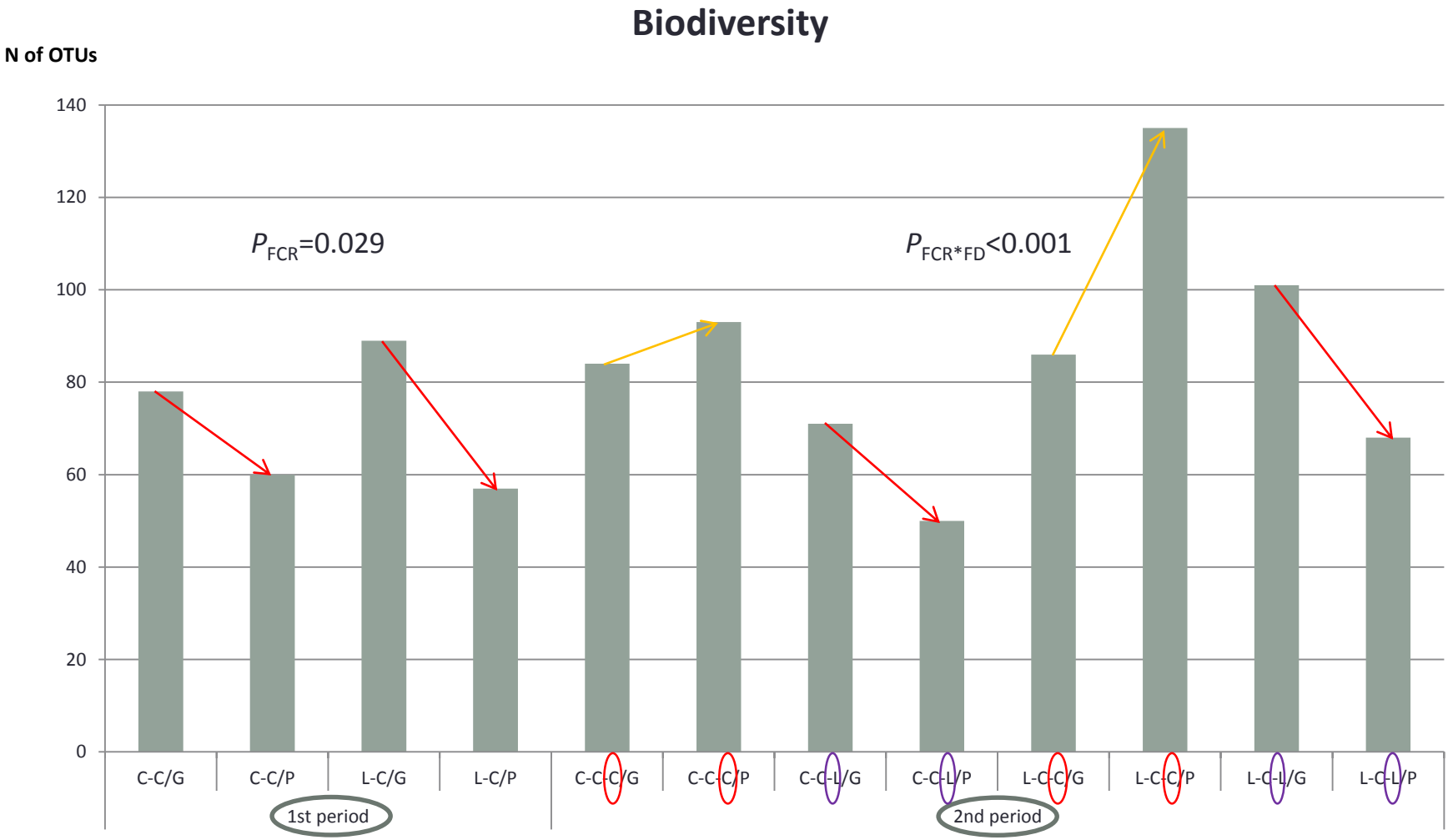
# Biodiversity Degree of Ileal Microbiota

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# Significant differences for Ileal Microbiota components related with FCR

| Kruskal-Wallis rank sum test & Feed Conversion Ratio | Average %GFC | Average %BFC | *P*-value |
|---|---|---|---|
| *Anaerostipes* | 1.66E-03 | ND | **0.027** |
| *Aerococcus* | 8.17E-05 | 1.43E-02 | **0.035** |
| *unclassified_Burkholderiales* | 1.61E-03 | 8.53E-04 | **0.044** |
| *unclassified_Lactobacillaceae* | 1.36E+00 | 1.23E+00 | 0.059 |
| *Brachybacterium* | 2.68E-04 | ND | 0.064 |
| *Curvibacter* | 2.04E-03 | ND | 0.064 |
| *Delftia* | 7.47E-04 | ND | 0.064 |
| *Parabacteroides* | 2.77E-03 | ND | 0.064 |

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# Significant differences for Ileal Microbiota components related with Diet

| Kruskal-Wallis rank sum test & Starter Diet | Average %IDC | Average %IDL | *P*-value |
|---|---|---|---|
| *Jeotgalicoccus* | ND | 1.30E-03 | **0.027** |
| *unclassified_Bacteroidetes* | 4.59E-03 | 2.90E-02 | **0.044** |
| *Catenibacterium* | ND | 1.51E-03 | 0.064 |
| *Comamonas* | 8.72E-04 | ND | 0.064 |
| *Deinococcus* | 1.45E-03 | ND | 0.064 |
| *Finegoldia* | ND | 3.88E-04 | 0.064 |
| *Peptoniphilus* | ND | 9.91E-04 | 0.064 |
| *unclassified_Bradyrhizobiaceae* | ND | 1.91E-04 | 0.064 |
| *Erysipelotrichaceae_incerta_sedis* | 1.44E-04 | 2.49E-03 | 0.074 |
| *Neisseria* | 8.17E-05 | 9.37E-04 | 0.097 |
| *unclassified_Rhodobacteraceae* | 6.89E-05 | 5.44E-04 | 0.097 |

| Kruskal-Wallis rank sum test & Finisher Diet | Average %FDC | Average %FDL | *P*-value |
|---|---|---|---|
| *Butyricicoccus* | 2.73E-02 | 4.33E-03 | **0.006** |
| *unclassified_Betaproteobacteria* | 8.37E-04 | ND | **0.011** |
| *unclassified_Clostridiales* | 4.42E-01 | 1.52E-01 | **0.016** |
| *unclassified_Clostridia* | 5.01E-03 | 1.86E-03 | **0.019** |
| *Bacteroides* | 2.30E-01 | 3.78E-02 | **0.02** |
| *Clostridium.XI* | 3.48E+00 | 1.44E+00 | **0.021** |
| *unclassified_Bacteria* | 2.22E+00 | 1.77E+00 | **0.021** |
| *unclassified_Lachnospiraceae* | 1.10E-01 | 2.50E-02 | **0.021** |
| *Methylobacterium* | 1.13E-03 | 1.50E-04 | **0.025** |
| *Streptophyta* | 1.28E-02 | 2.30E-03 | **0.027** |
| *Cloacibacterium* | ND | 2.84E-04 | **0.027** |
| *Fusobacterium* | ND | 2.68E-04 | **0.027** |
| *Ruminococcus* | 1.71E-03 | ND | **0.027** |
| *unclassified_Peptostreptococcaceae* | 1.81E-01 | 8.99E-02 | **0.027** |
| *Clostridium.IV* | 2.47E-03 | 2.60E-03 | **0.037** |
| *Sphingomonas* | 7.70E-04 | ND | **0.043** |
| *unclassified_Firmicutes* | 8.32E-01 | 7.56E-01 | **0.046** |
| *unclassified_Ruminococcaceae* | 9.29E-02 | 2.32E-02 | **0.046** |
| *Barnesiella* | 2.76E-03 | 1.50E-04 | **0.046** |
| *Alistipes* | 1.13E-02 | 2.74E-03 | 0.061 |
| *Brachybacterium* | ND | 1.44E-04 | 0.064 |
| *Coprobacillus* | 6.38E-04 | ND | 0.064 |
| *Coprococcus* | 1.39E-03 | ND | 0.064 |
| *Gemella* | ND | 9.26E-04 | 0.064 |
| *Granulicatella* | 2.42E-03 | ND | 0.064 |
| *Megasphaera* | 2.66E-03 | ND | 0.064 |
| *Peptoniphilus* | ND | 1.66E-03 | 0.064 |
| *Clostridium.XlVb* | 2.32E-02 | 2.97E-03 | 0.073 |
| *unclassified_Bacteroidales* | 1.30E-02 | 2.85E-03 | 0.073 |
| *Sarcina* | 1.21E-02 | 1.44E-04 | 0.074 |
| *Pelomonas* | 4.39E-02 | 1.05E-02 | 0.074 |
| *unclassified_Comamonadaceae* | 6.31E-03 | 2.84E-03 | 0.093 |
| *Actinomyces* | 7.37E-04 | ND | 0.097 |

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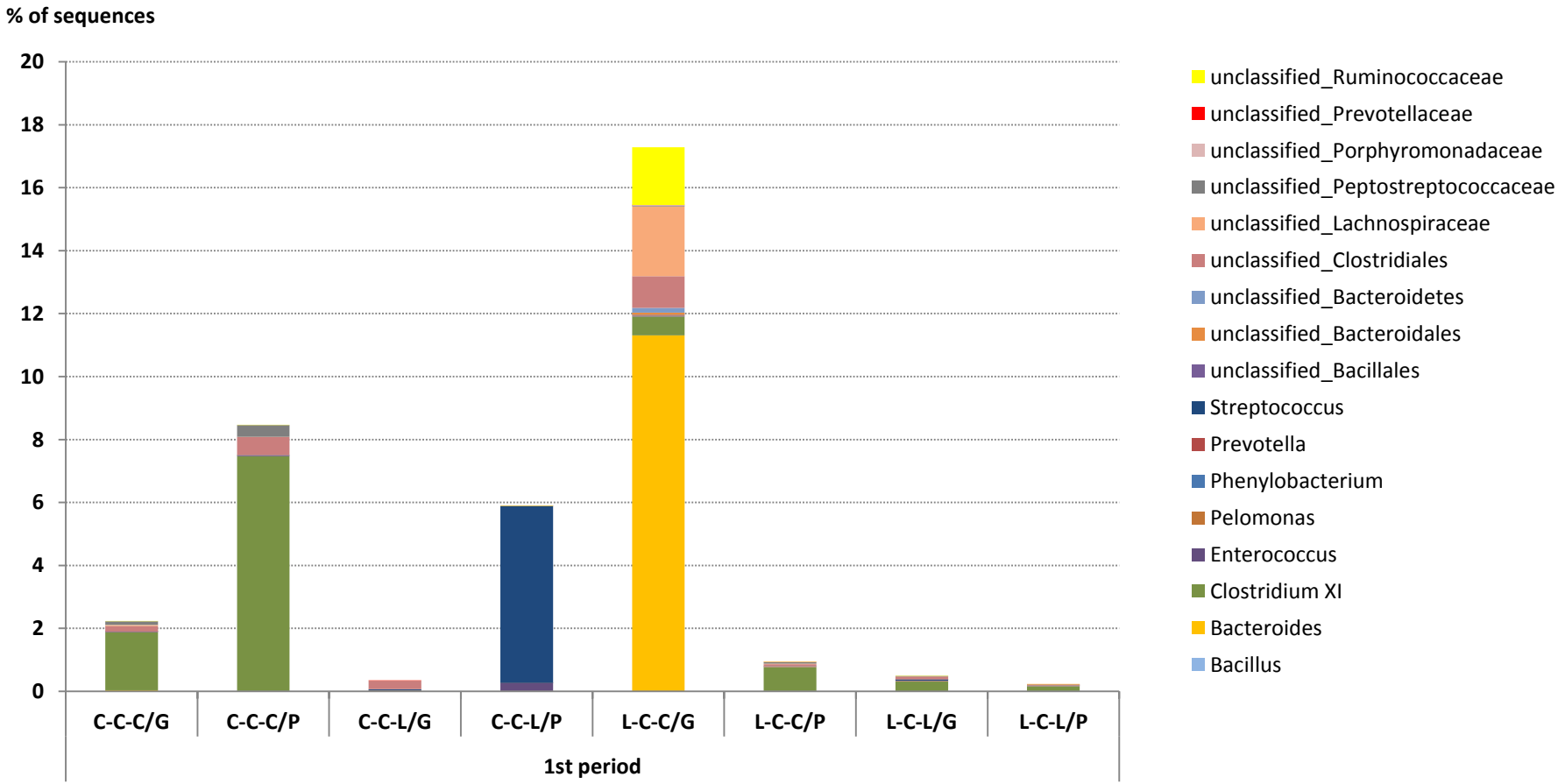
# Principal Ileal Microbiota components without *Lactobacillus*

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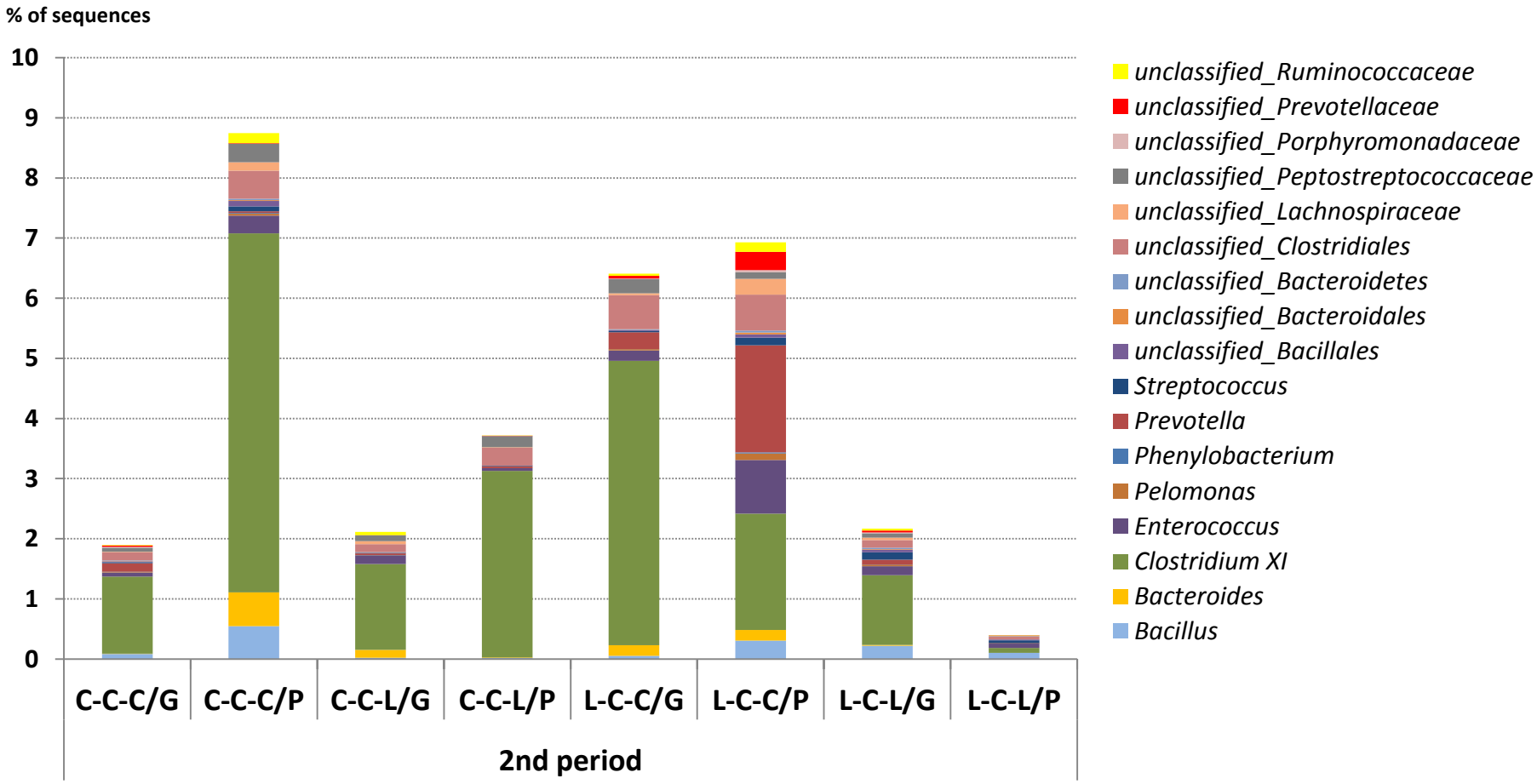
# Principal Ileal Microbiota components without *Lactobacillus*

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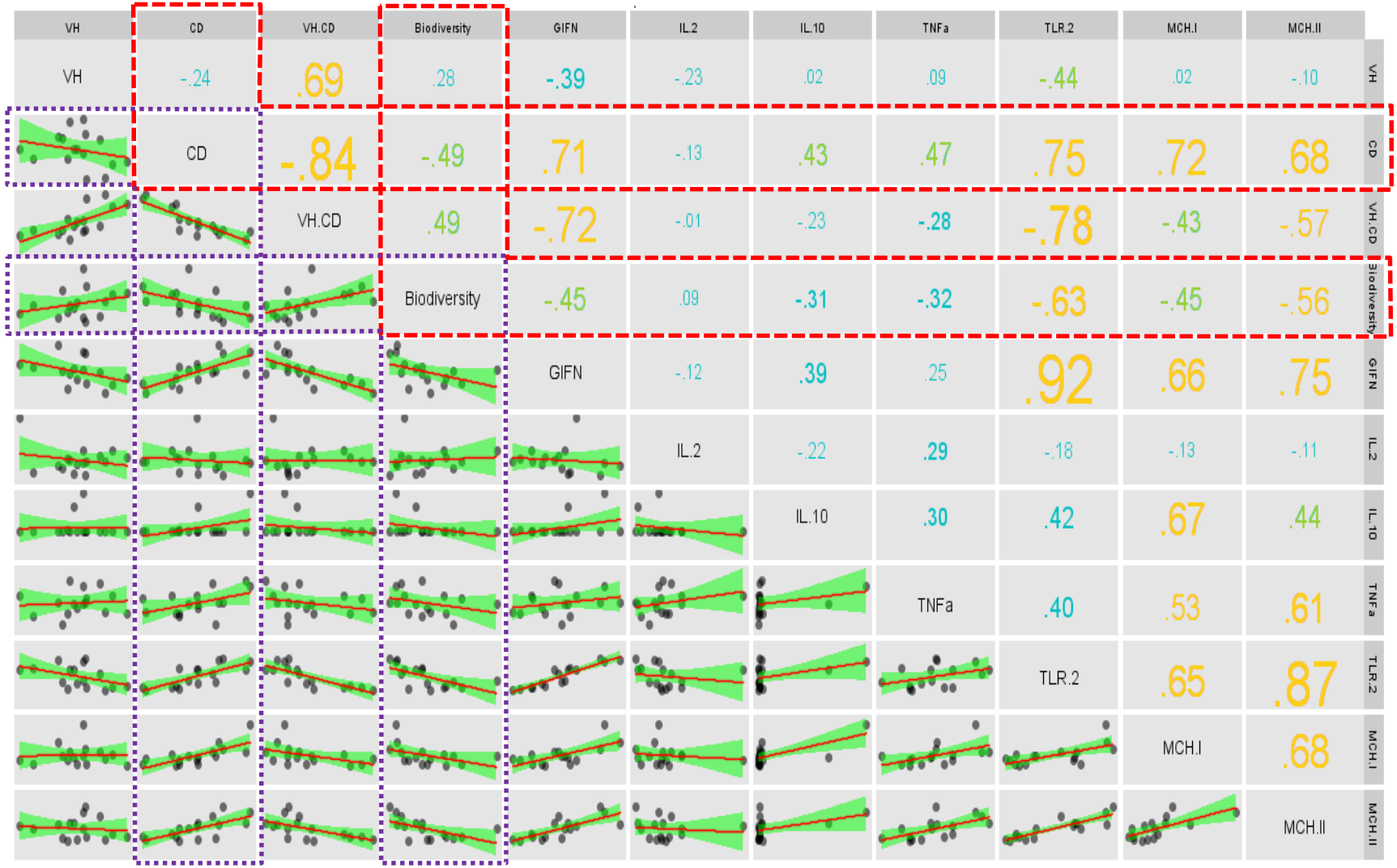
# Pearson's product-moment correlation




P<0.05

0.1>P>0.05

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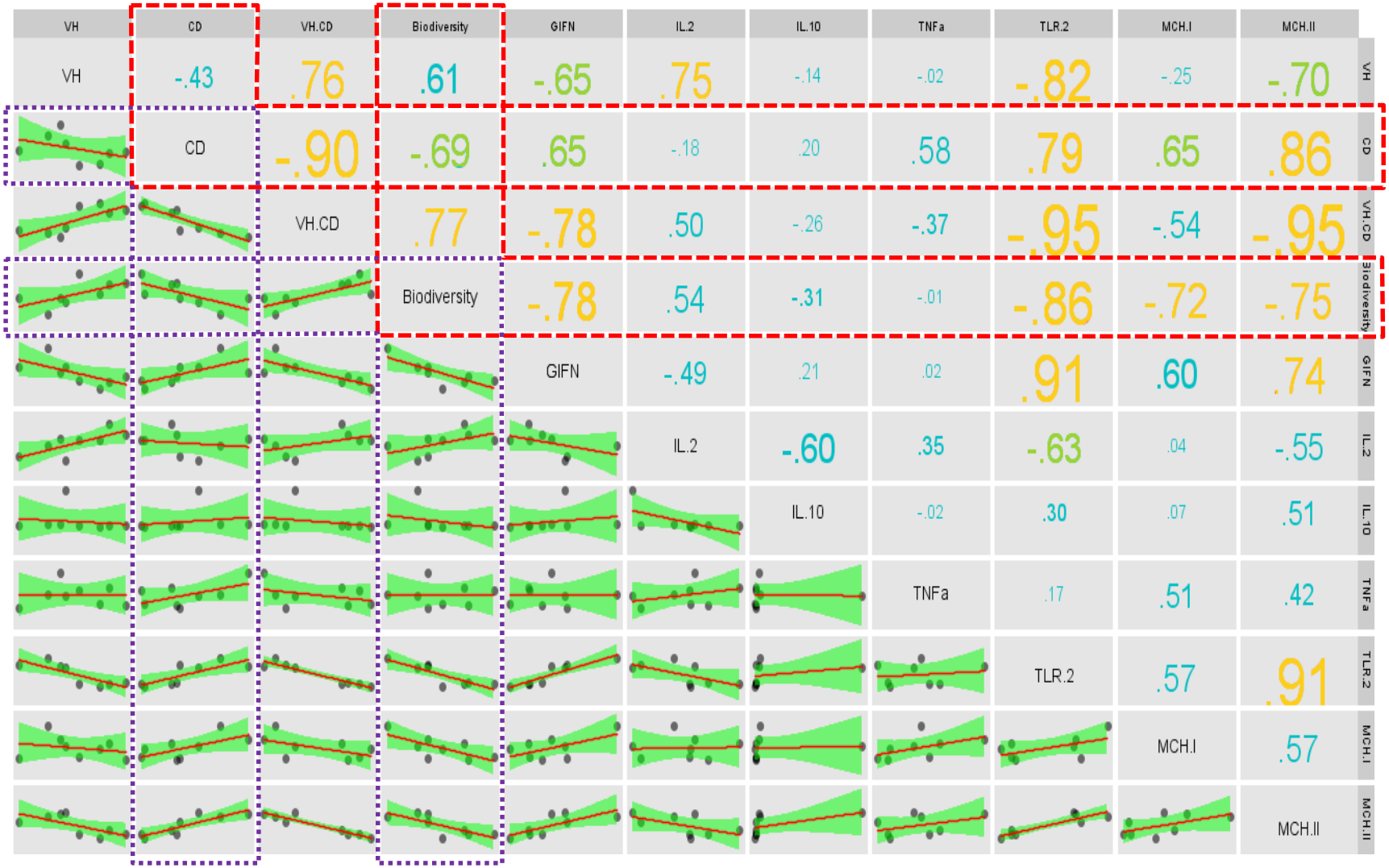
# Pearson's product-moment correlation (Good FCR)




P<0.05

0.1>P>0.05

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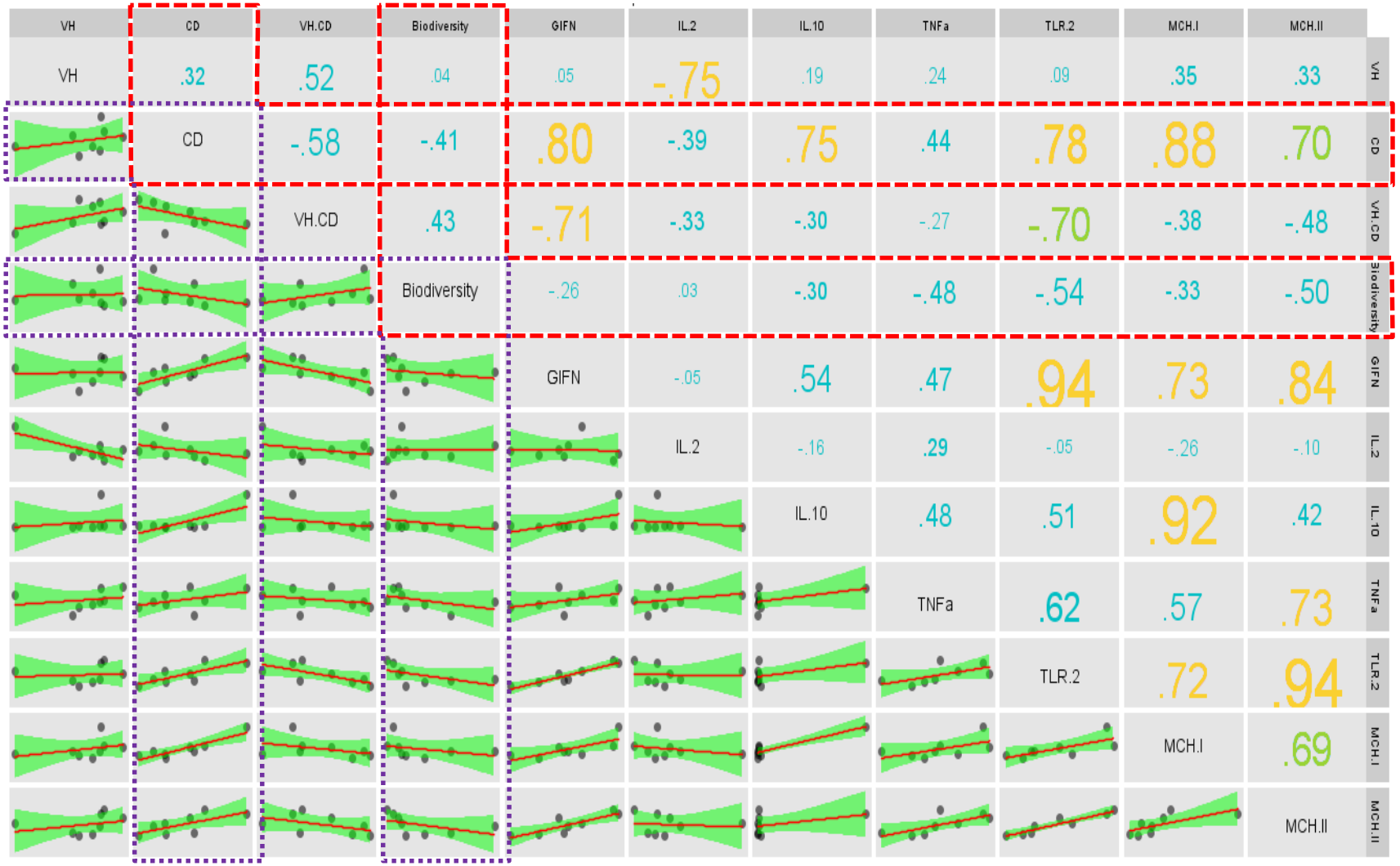
# Pearson's product-moment correlation (Poor FCR)

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0.1>P>0.05

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# CONCLUSIONS

- During the second period of the trial, significant effect on intestinal morphology was observed for diet and FCR.
- Correlations (tendency) between some immunological parameters and FCR have been obtained: poor FCR higher inflammatory response.
- Significant differences of intestinal microbiota were obtained in relation with diet changes and FCR.
- Significant correlations between intestinal morphology, intestinal microbiota biodiversity and immunological parameters of intestinal mucosa were obtained.

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- Miguel Algaba and Liliana Signeanu

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# TRIAL Nº B-443 (IRTA)
# ECO-FCE PROJECT. TRIAL Nº1
# WP2: IDENTIFICATION AND OPTIMISATION OF FEED STRATEGIES

## THANKS FOR YOUR ATTENTION

IRTA-CReSA

Results of Intestinal Microbiota (deep sequencing), Intestinal Mucosa Histology, Intestinal Mucosa Immunological Parameters

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