1 **Leveraging host-genetics and gut microbiota to determine immunocompetence in pigs**
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20 **Abstract**
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22   The aim of the present work was to identify microbial biomarkers linked to
23 immunity traits and to characterize the contribution of host-genome and gut microbiota to
24 the immunocompetence in healthy pigs. To achieve this goal, we undertook a combination
25 of network, mixed model and microbial-wide association studies (MWAS) for 21
26 immunity traits and the relative abundance of gut bacterial communities in 389 pigs
27 genotyped for 70K SNPs. The heritability (h^2; proportion of phenotypic variance explained
28 by the host genetics) and microbiability (m^2; proportion of variance explained by the
29 microbial composition) showed similar values for most of the analyzed immunity traits,
30 except for both IgM and IgG in plasma that were dominated by the host genetics, and the
31 haptoglobin in serum which was the trait with larger m^2 (0.275) compared to h^2 (0.138).
32 Results from the MWAS suggested a polymicrobial nature of the immunocompetence in
33 pigs and revealed associations between pigs gut microbiota composition and 15 of the
34 analyzed traits. The lymphocytes phagocytic capacity (quantified as mean fluorescence)
35 and the total number of monocytes in blood were the traits associated with the largest
36 number of taxa (6 taxa). Among the associations identified by MWAS, 30% were
37 confirmed by an information theory network approach. The strongest confirmed

38 associations were between *Fibrobacter* and phagocytic capacity of lymphocytes (r=0.37),

39 followed by correlations between *Streptococcus* and the percentage of phagocytic

40 lymphocytes (r=-0.34) and between *Megasphaera* and serum concentration of haptoglobin

41 (r=0.26). In the interaction network, *Streptococcus* and percentage of phagocytic

42 lymphocytes were the keystone bacterial and immune-trait, respectively. Overall, our

43 findings reveal an important connection between immunity traits and gut microbiota in

44 pigs and highlight the need to consider both sources of information, host genome and

45 microbial levels, to accurately characterize immunocompetence in pigs.

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47
48 **Introduction**

49   The pig industry has a considerable socio-economical value representing around

50 35% of the total meat produced worldwide [1] and being the most popular meat for

51 consumption [2]. The intensification of pig production coupled with the ban on in-feed use

52 of antibiotics has led to a deterioration of the health status of pig farms. In addition, the

53 current emergence of antibiotic resistance and society demands for healthier products and

54 environmentally responsible livestock systems, has motivated to explore relevant

55 approaches for pig and other livestock breeding programs, to improve robustness and

56 disease resistance [3].

57   The implementation of breeding programs to select animals according to their

58 robustness presents several challenges and levels of complexity. One of the most relevant

59 milestones is the identification of selection criteria that combine functional traits with

60 those of immunocompetence. These complex traits are driven by several physiological and

61 behavioral mechanisms that in turn are determined by genetic and environmental factors.

62 Regarding the genetic determinism of immunocompetence, several studies in pigs

63 acknowledged medium to high heritability estimates [4-9] and reported genomic regions

64 and candidate genes associated with phenotypic variation of health-related traits [9-15].

65   Over the past few years, multiple studies highlighted the relevant role of the gut

66 microbiota composition in the homeostasis and function of the mammalian immune system

67 [16-19]. Gut microbiota can regulate host-immunity through both direct mechanisms like

68 translocation of bacteria and their components (i.e. metabolites), or mediate indirect process

69 such as T-cell polarization and the regulation of immune cell trafficking [18]. Commensal

70 gut populations modulate hosts' immune responses, which in turn can modify the

71 microbiota composition to maintain gut homeostasis [20, 21]. Recently, polymorphisms
72 located in immune genes associated with the abundance of microbial communities have
73 been reported [22-25]. Furthermore, it has been suggested that the pattern recognition
74 receptors, which are proteins capable of recognizing molecules frequently associated with
75 pathogens, may have evolved to mediate the bidirectional crosstalk between microbial
76 symbionts and their hosts [26]. This has resulted in a mutualistic and symbiotic partnership
77 between the immune system and these commensal microorganisms [27]. Therefore, the
78 immune system not only protects the host from pathogens but can also modulate, and is
79 itself modulated, by beneficial microbes.

80   Considering the relevant interplay between gut microbiota and host immunity, a
81 better understanding of the role of gut microbiota in the immunocompetence determination
82 in pigs could greatly assist in the implementation of selection programs to improve
83 robustness and disease resistance simultaneously. The present work aimed to identify
84 microbial biomarkers linked to immunity traits and to estimate the contribution of host-
85 genome and gut microbial communities to the immunocompetence in healthy pigs.

86 **Material and Methods**

87 **Ethics Statement**

88   All experimental procedures were performed according to the Spanish Policy for
89 Animal Protection RD53/2013, which meets the European Union Directive 2010-63-EU
90 about the protection of animals used in experimentation. The experimental protocol was
91 approved by the Ethical Committee of the Institut de Recerca i Tecnologia
92 Agroalimentàries (IRTA).

93 **Animal Samples**

94   Samples employed in this study are a subset of pigs reported in Ballester et al. [9]
95 and Reverter et al. [25]. A total of 405 weaned piglets (204 males and 201 females) from a
96 commercial Duroc pig line were used. The pigs were distributed in six batches obtained
97 from 132 sows and 22 boars. All animals were raised on the same farm and fed *ad libitum*
98 a commercial cereal-based diet.

99 **Immunity and hematological traits**

100   Details of the sampling and laboratory processing have been reported [9]. In brief,
101   blood and saliva samples were collected from all 405 piglets at 60 ± 8 days of age. Blood
102   samples in 4 ml EDTA tubes were used to measure the hemograms (Laboratory
103   Echevarne, Spain; Barcelona). Saliva was collected with Salivette tubes (Sarstedt S.A.U.,
104   Germany) according to the protocols recommended by the manufacturer. Blood samples
105   for serum were collected in 6 mL tubes with gel serum separator and centrifuged at 1600 g
106   for 10 min at RT. Plasma was collected from the sampled blood in 6 ml heparinized tubes
107   and centrifuged at 1300 g for 10 minutes at 4ºC. Plasma and serum samples were collected,
108   aliquoted, and stored a -80ºC. The following haematological parameters were included in
109   this study: total number of eosinophils (EO), leukocytes (LEU), lymphocytes (LYM) and
110   neutrophils (NEU) in blood. Analyzed immunity parameters included immunoglobulins
111   (IgA, IgG and IgM) concentrations in plasma; C-reactive protein (CRP), Haptoglobin (HP)
112   and Nitric Oxide (NO) concentrations in serum; and IgA concentration in saliva (IgAsal).
113   Gamma-delta T cells ($\gamma\delta$ T cells) were separated from heparinised peripheral blood by
114   density-gradient centrifugation with Histopaque-1077 (Sigma, Spain). Phagocytosis assay
115   was carried out in heparinized whole blood samples incubated with fluorescein (FITC)-
116   labelled opsonized *Escherichia coli* bacteria using the Phagotest kit (BD Pharmigen,
117   Spain) as indicated in the manufacturer's protocol. The following phagocytosis traits were
118   used: percentage of total phagocytic cells (PHAGO_%); percentage of phagocytic cells
119   among granulocytes (GRANU_PHAGO_%), monocytes (MON_PHAGO_%) and
120   lymphocytes (LYM_PHAGO_%); mean fluorescence in FITC among the total phagocytic
121   cells (PHAGO_FITC); and mean fluorescence in FITC among the granulocytes (GRANU_
122   PHAGO_FITC), monocytes (MON_PHAGO_FITC) and lymphocytes
123   (LYM_PHAGO_FITC) that phagocyte.

124
125   **DNA extraction, sequencing and bioinformatics analysis**
126
127   Simultaneous with blood and saliva samples, fecal samples were collected from all
128   405 piglets. DNA was extracted with the DNeasy PowerSoil Kit (QIAGEN, Hilden,
129   Germany) following manufacturer's instructions. Extracted DNA was sent to the
130   University of Illinois Keck Center for Fluidigm sample preparation and paired-end (2 ×
131   250 nt) sequencing on an Illumina NovaSeq (Illumina, San Diego, CA, USA). The 16S
132   rRNA gene fragment was amplified using the primers V3_F357_N: 5'-
133   CCTACGGGNGGCWGCAG-3' and V4_R805: 5'-GACTACHVGGGTATCTAATCC-3'.

4

134 Sequences were analysed with *Qiime2* [28]; barcode sequences, primers and low-quality

135 reads (Phred scores of <30) were removed. The quality control also trimmed sequences

136 based on expected amplicon length and removed chimeras. Afterwards, sequences were

137 processed into Amplicon Sequences Variants (ASVs) at 99% of identity. Samples with less

138 than 10,000 reads were excluded and ASVs present in less than three samples and

139 representing less than 0.001% of the total counts were discarded. ASVs were classified to

140 the lowest possible taxonomic level based on a primer-specific trained version of

141 GreenGenes Database [29].

142

143 **Genotype data**

144 A total of 390 out of 405 animals were genotyped using the Porcine 70 k GGP

145 Porcine HD Array (Illumina, San Diego, CA) containing 68,516 single nucleotide

146 polymorphisms (SNPs). The quality control excluded SNPs with minor allele frequencies

147 < 5%, rates of missing genotypes above 10%, and SNPs that did not map to the porcine

148 reference genome (Sscrofa11.1 assembly). Consequently, 42,641 SNPs were retained for

149 subsequent analysis.

150 **Microbiability and heritability estimation**

151 Heritability (h^2), i.e. the proportion of variance explained by the host genetics, and

152 microbiability (m^2), i.e. the proportion of variance explained by the microbial composition,

153 were estimated for each immunity trait based on a mixed-model as follows:

$$y = X\beta + Zu + Wm + e$$

155 where y is the n-dimensional vector containing the individual phenotypes for the immune

156 trait under consideration; β is the vector of fixed effects, containing the general intercept,

157 the sex effect (two levels), and batch effect (six levels) for most traits but data of

158 laboratory analysis (12 levels, two by batch) for phagocytosis-related traits; u is the vector

159 containing the host genetic random effect from each individual; m is the vector of the

160 animal's microbiome random effect; X, Z and W are, respectively, the incidence matrices

161 correspondent to β, u and m; and e is the vector of residual terms.

162 Assuming independence between random effects, the following distributions were

163 considered: $u \sim N(0, G, \sigma^2_u)$, where σ^2_u is the host genetic effects variance and G is the

164 genomic relationship matrix between individuals, computed following [30], i.e.,

165 $G = \dfrac{SS'}{2\sum_i p_i(1-p_i)}$ being S the matrix that contains the centered individual genotype for the

5

166   42,641 SNPs (columns) of each individual (rows), and p_j is the frequency of the minimum

167   allele of the jth SNP; $m \sim N(0, B, \sigma^2_m)$, where σ^2_m is the microbial effects variance and **B** the

168   microbial relationship matrix computed following [31], i.e., $B = \frac{MM'}{n}$, being **M** the matrix

169   containing the scaled after a previous cumulative sum scaling normalization of the   ASV

170   abundances (columns) for each individual microbiome (rows) and n the total number of

171   ASVs; and finally $e \sim N(0, I\sigma^2_e)$, where σ^2_e is the error variance.

172

173   The model parameters for each immunity trait were estimated by a Bayesian

174   approach, using the Bayes Ridge Regression model from BGLR package [32]. We used a

175   Gibbs sampler with 30,000 iterations and a burn-in of 3,000 rounds. The 'heritability'

176    $\left(h^2 = \frac{\sigma^2_u}{(\sigma^2_u + \sigma^2_m + \sigma^2_e)}\right)$ and 'microbiability' $\left(m^2 = \frac{\sigma^2_m}{(\sigma^2_u + \sigma^2_m + \sigma^2_e)}\right)$ were estimated from the

177   mean   of the posterior distributions [33].

178

179   **Microbial Wide Association Study**

180   We performed a Microbial Wide Association Study (MWAS) using a multi-ASV

181   association method that combines all the ASVs in a single model:

182

$$y_i = \beta_0 + \sum_{j \in 1..p} \beta_j x_{ij} + \epsilon_i \ (2)$$

183

184

185   Given a trait y_i measured in n individuals and a matrix **X** containing relative abundances

186   of p taxa from a microbial community, here the ASVs effects were treated as draws from

187   normal distributions as in any Bayesian Ridge Regression approach [32].

188

189   Following the approach of Legarra et al [34], Bayes Factor (BF) for the effect of each taxa

190   can be derived as the ratio of probabilities $BF = \frac{P_{H1}(y)}{P_{H0}(y)}$, where H1 means "the j-genus has

191   some effect" and H0 "the j-genus has no effect". The calculations from the posterior

192   distribution are very simple since both probabilities (P_{H1}, P_{H0}) are normal density.

193

194   **Network between microbial and immunity traits**

195   To better understand the relationship between microbial communities and

196   immunity traits we implemented PCIT [35], a network-based approach that combines

197 partial correlation coefficient with information theory to identify significant correlations
198 between each possible combination of clr-transformed bacterial abundance and the
199 immune-traits [35]. PCIT tests all possible 3-way combinations in the dataset and only
200 keeps correlations between traits if they are significant and independent of the association
201 of another features. To reduce the complexity of the resulting network, from the PCIT
202 significant connections, we kept only the ones involving one immune-trait and one genus
203 (i.e. genus-genus and trait-trait interactions were no represented).

204

205 **Results**

206   In this study, 16S rRNA gene sequences, host genotype information and immune
207 traits from 389 Duroc pigs were analyzed to estimate both host genomes and gut
208 microbiota contribution to the porcine immunocompetence, and to identify microbial
209 biomarkers linked to immunity traits. Table 1 summarizes the immunity traits and their
210 descriptive statistics used in the present study. Regarding 16S rRNA gene sequences, after
211 quality control, a total of 2,055 Amplicon Sequences Variants (ASVs) and 68 genera were
212 detected. The dominant bacterial phyla were Bacteroidetes and Firmicutes, and the most
213 abundant genera were *Prevotella, Lactobacillus, Treponema, Roseburia* and *Ruminococcus*
214 (Supplementary figure 1)

215

216 **Table 1**. Descriptive statistics, mean, standard deviation (SD) and coefficient of variation
217 (CV) of the 21 analysed traits.
218
219

Trait	Abrev	Mean	SD	CV
C-reactive protein in serum (µg/ml)	CRP	176.69	128.61	0.73
Eosinophils count n/µL	EO	406.31	191.04	0.47
γδ T-lymphocyte subpopulation (%)	γδ T-cells	7.83	4.94	0.63
Granulocytes phagocytosis FITC	GRANU_PHAGO_FITC	5.15	0.42	0.08
Granulocytes phagocytosis (%)	GRANU_PHAGO_%	91.70	3.85	0.04
Haptoglobin in serum (mg/ml)	HP	1.03	0.67	0.65
IgA in plasma (mg/ml)	IgA	0.65	0.31	0.48
IgA in saliva (mg/dl)	IgAsal	5.13	3.12	0.61
IgG in plasma (mg/ml)	IgG	12.64	4.85	0.38
IgM in plasma (mg/ml)	IgM	2.27	0.79	0.35
Leukocytes count n/µL	LEU	20,350.15	6948.01	0.34
Lymphocytes count n/µL	LYM	12,422.40	4497.03	0.36
Lymphocytes phagocytosis FITC	LYM_PHAGO_FITC	3.16	0.13	0.04

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Lymphocytes phagocytosis (%)	LYM_PHAGO_%	6.06	4.02	0.66
Monocytes phagocytosis FITC	MON_PHAGO_FITC	3.92	0.25	0.06
Monocytes phagocytosis (%)	MON_PHAGO_%	49.48	9.72	0.20
Monocytes count n/μL	MONOCITOS_MM	548.42	280.43	0.51
Nitric oxide in serum (μM)	NO	206.45	79.83	0.39
Phagocytosis FITC	PHAGO_FITC	4.71	0.33	0.07
Phagocytosis (% cells)	PHAGO_%	42.94	8.37	0.19
Neutrophils count n/μL	NEU	6985.62	3316.61	0.47

220
221

Heritability and microbiability of immunity traits

Posterior estimates of h^2 and m^2 for the 21 health-related traits can be shown in Figure 1 and Supplementary Table 1. Posterior means of h^2 in the analyses considering microbiota contribution reached low to medium values (from 0.138 to 0.359), but posterior probability of h^2 being superior to 0.1 was in all cases above 0.82. Similarly, estimated m^2 reached values between 0.152 and 0.276, and the probability of being above 0.1 was above 0.85 for all immunity and hematological traits (Supplementary Table 1). Among analysed traits, IgG and IgM in plasma showed the highest genetic determinism (h^2= 0.316 and 0.359), whereas microbiota contribution was below 0.18. Conversely, the Hp concentration in serum showed the highest microbial effect (m^2=0.276), accompanied by the lowest h^2 estimate (h^2= 0.138). Considering the joint effects of host-genome and gut microbiota, these two sources of variation explained from 29.9% to 51.7% phenotypic variance of the analysed immunity and hematological traits. To be noted, in the 76% (16/21) of these traits the h^2 and m^2 estimates reaching relatively similar values (Figure 1).

Figure 1. Percentage of phenotypic variance explained by the host-genetic (red points) and the gut microbial composition (green points) for most relevant immunity traits.
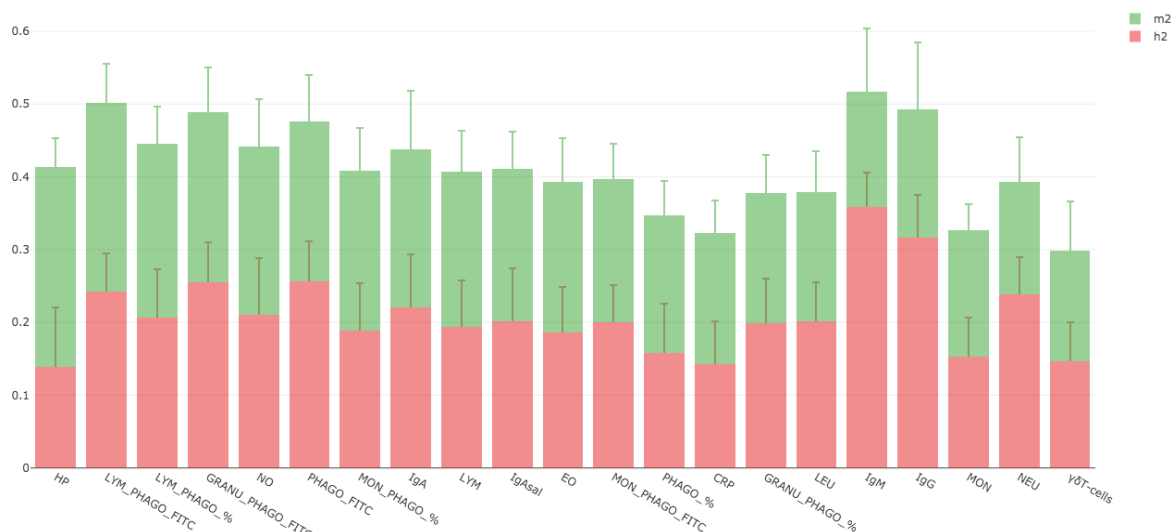
Associations between microbial genera and immunity traits

Results from the MWAS reported some putative associations between bacterial genera abundance and health-related traits (Table 2). In particular, 15 out of the 21 immunity traits were associated with at least one microbial genus (Table 2). The strongest association was observed between the relative abundance of *Chlamydia* and the profile of LYM_PHAGO_FITC, followed by *Streptococcus* linked to LYM_PHAGO_% and *Peptococcus* associated with LYM_PHAGO_FITC. In addition, several genera showed multiple associations with numerous immunity traits: *Desulfovibrio, Oribacterium* and *Chlamydia* (4 traits) followed by *Oxalobacter* and *Parabacteroides* (3 traits), *Peptococcus* and *Streptococcus* (2 traits). As far as the analysed phenotypes, those traits showing the highest number of associations with different bacterial taxa were: LYM_PHAGO_FITC and MON (6 taxa); LYM_PHAGO_%, EOS, GRANU_PHAGO_FITC (4 taxa) and total number of LEU (3 taxa). Meanwhile, only four out of the 15 immunity traits analysed were linked with only one genus (Table 2).

Table 2. Results from the microbial-wide association studies

Names	Description_trait	Genus	BPcum
MON	Monocytes count n/μL	*Anaerobiospirillum*	2.676
LYM_PHAGO_FITC	Lymphocytes phagocytosis FITC	*Bacteroides*	2.536
IgG	IgG in plasma (mg/ml)	*Bulleidia*	2.313
GRANU_PHAGO_FITC	Granulocytes phagocytosis FITC	*Campylobacter*	6.556
MON	Monocytes count n/μL	*Catenibacterium*	2.952
EO	Eosinophils count n/μL	*Chlamydia*	5.292
IgAsal	IgA in saliva (mg/dl)	*Chlamydia*	2.364
LYM_PHAGO_FITC	Lymphocytes phagocytosis FITC	*Chlamydia*	11.246

9

LYM_PHAGO_%	Lymphocytes phagocytosis (%)	*Chlamydia*	3.088
EO	Eosinophils count n/μL	*Desulfovibrio*	3.369
LEU	Leukocytes count n/μL	*Desulfovibrio*	2.085
LYM_PHAGO_FITC	Lymphocytes phagocytosis FITC	*Desulfovibrio*	2.852
MONOCITOS_MM	Monocytes count n/μL	*Desulfovibrio*	2.427
LYM_PHAGO_FITC	Lymphocytes phagocytosis FITC	*Fibrobacter*	3.829
HP	Haptoglobin in serum (mg/ml)	*Megasphaera*	3.582
MON_PHAGO_FITC	Monocytes phagocytosis FITC	*Mitsuokella*	2.788
CRP	C-Reactive Protein in serum (ug/ml)	*Mucispirillum*	2.588
LYM_PHAGO_%	Lymphocytes phagocytosis (%)	*Oribacterium*	3.089
MON_PHAGO_FITC	Monocytes phagocytosis FITC	*Oribacterium*	2.673
PHAGO_%	% of total phagocytic cells	*Oribacterium*	2.009
NEU	Neutrophils count n/μL	*Oribacterium*	2.478
LEU	Leukocytes count n/μL	*Oxalobacter*	2.816
MON	Monocytes count n/μL	*Oxalobacter*	2.320
NEU	Neutrophils count n/μL	*Oxalobacter*	3.216
IgG	IgG in plasma (mg/ml)	*Paludibacter*	3.018
HP	Haptoglobin in serum (mg/ml)	*Parabacteroides*	2.018
LYM_PHAGO_FITC	Lymphocytes phagocytosis FITC	*Parabacteroides*	2.219
MON_PHAGO_FITC	Monocytes phagocytosis FITC	*Parabacteroides*	5.158
LYM_PHAGO_FITC	Lymphocytes phagocytosis FITC	*Peptococcus*	7.497
PHAGO_%	% of total phagocytic cells	*Peptococcus*	2.288
GRANU_PHAGO_FITC	Granulocytes phagocytosis FITC	*rc4.4*	2.156
LEU	Leukocytes count n/μL	*rc4.4*	2.619
LYM	Lymphocytes count n/μL	*rc4.4*	3.621
MON	Monocytes count n/μL	*RFN20*	3.022
GRANU_PHAGO_FITC	Granulocytes phagocytosis FITC	*Sphaerochaeta*	2.686
γδ T-cells	γδ T-Lymphocyte subpopulation	*Streptococcus*	2.444
LYM_PHAGO_%	Lymphocytes phagocytosis (%)	*Streptococcus*	8.484
EO	Eosinophils count n/μL	*Succinivibrio*	4.814
MON	Monocytes count n/μL	*Succinivibrio*	2.439
LYM_PHAGO_%	Lymphocytes phagocytosis (%)	*Treponema*	2.187

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Gut microbial and host-immune interaction network

The interplay between microbial and health-related traits was also inferred through a network comprised of 63 nodes (42 genera and 21 immunity traits) and 86 edges (significant connections) in which only the significant interactions between a bacterial genus and an immunity trait were considered (Figure 2). The topological evaluation of the network highlights LYM_PHAGO_% as the most connected trait, followed by IgAsal, NEU and Hp. Meanwhile, at microbial level, *Streptococcus* was the most connected genus followed by *Acidaminococcus, Desulfovibrio* and *Blautia*. The network approach

10

267   confirmed 30% (12/40) of the associations identified by the MWAS (Figure 2). The

268   strongest confirmed correlation was between *Fibrobacter* and LYM_PHAGO_FITC (r=

269   0.37) followed by correlations between *Streptococcus* and LYM_PHAGO_% (r=-0.34) and

270   between *Megasphaera* and Hp (r=0.26). To be noted, *Streptococcus* and

271   LYM_PHAGO_% that showed the strongest confirmed association in the MWAS were

272   highly in the interaction-network as the keystone bacterial and immunity trait, respectively.

273

274   **Figure 2**. Microbial - health-related traits network. Green diamond nodes correspond to

275   immunity traits (n=21) and blue ellipse nodes correspond to microbial genera (n=42). Node

276   sizes are relative to their topological degree (number of connections) and edges are

277   continuous or dashed to represent positive or negative correlations, respectively.

278   Relationships previously identified by MWAS are highlighted in red.

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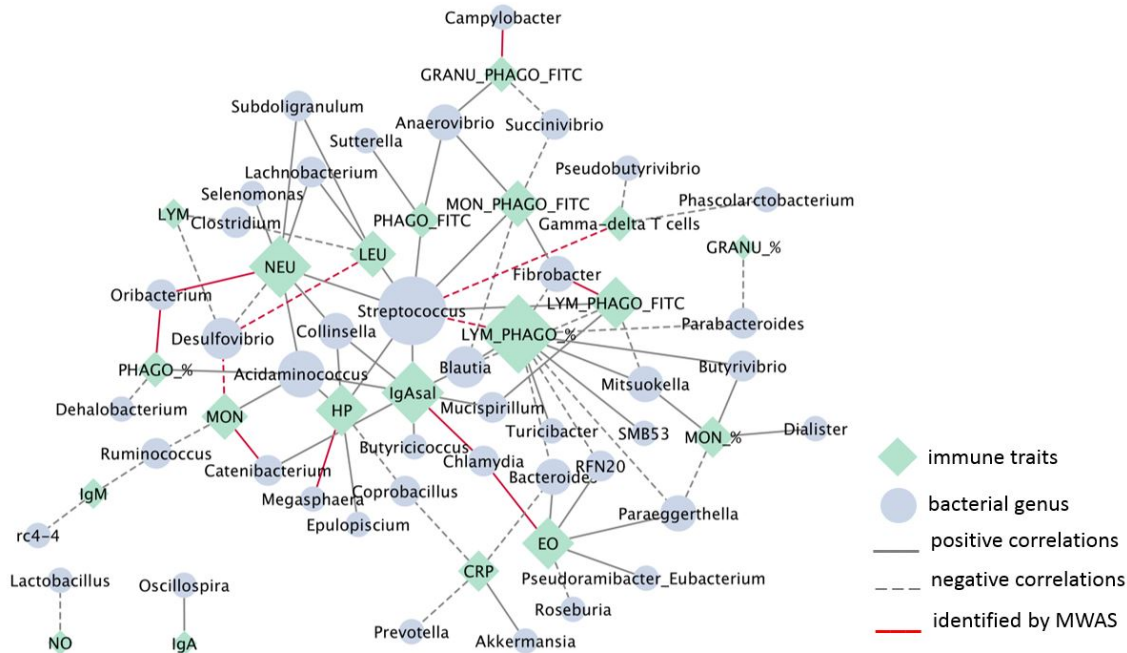
284   **General Discussion**

285   **Host-genome and gut microbiota contribution to porcine immunocompetence**

286   We report the first study that aimed to dissect the joint contribution of the host

287   genome and the gut microbiota to the immunocompetence in healthy pigs. Estimates of

288   microbiability pointed out significant microbial effects on most immunity and

289   hematological traits, ranging between 15% and 27% of total phenotypic variance. Effects

11

290 of microbiota resulted particularly relevant for Hp concentrations in serum, followed by

291 the parameters related to phagocytosis of lymphocytes. Regarding genomic heritabilities of

292 these traits, they reached low to moderate values and were substantially lower compared to

293 the medium to high h^2 previously obtained in the same Duroc population [9] for all traits

294 but MON and MON_PHAGO_%. A dramatic decrease of the estimated host genetic

295 effects was observed for γδ T cells, but also for EOS and NEU counts and

296 immunoglobulins concentrations in plasma, despite IgM and IgG variability seemed

297 dominated by host genetics and showed the highest h^2 among analysed traits. These results

298 would call into question the high genetic determinism of the global immunocompetence in

299 pigs reported in previous studies [6, 7, 9]. However, it should be considered that the

300 limited sample size joint with the likely similarity between close relatives (particularly

301 between full-sibs) in their microbiota profiles makes plausible that the model could not

302 separate adequately genetic from microbiota effects.

303

304 **Microbial signatures associated with immunity traits**

305 In the present study, we implemented a combination of MWAS and network

306 approaches to pinpoint microbial signatures associated with immunity traits, revealing

307 some interesting associations between the composition of the pig gut microbiota and the

308 host immunity traits. Remarkably, lymphocyte phagocytosis traits were among the most

309 connected and associated traits to the highest number of taxa and were also central nodes

310 in the network. The strongest confirmed association involved *Fibrobacter* relative

311 abundance in gut microbiota and the host phagocytosis capacity of lymphocytes, which

312 were positively correlated (r=0.37). *Fibrobacter* genus is composed of strictly anaerobic

313 bacteria with cellulolytic capacity capable of degrading complex plant fiber [36] and it has

314 been associated with better feed efficiency in pigs [37, 38]. Conversely, the relative

315 abundance of *Streptococcus* showed an opposite association with the percentage of

316 phagocytic lymphocytes (r=-0.34). *Streptococcus* was also the keystone taxa in the

317 network. In pigs, some *Streptococcus* species are important opportunistic pathogens such

318 as *Streptococcus suis*, which abundance increased in the stomach and small intestine after

319 weaning [39]. Piglets with high intestinal concentrations of *S. suis* can serve as a source of

320 transmission and infection between animals and farms (reviewed in [39]). In general

321 *Streptococcus* are less abundant in more-feed efficient pigs [37], although there are also

322 evidences of the immunomodulatory properties of member of *Streptococcus* genus [40,

323 41].

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324   Several studies in mammals have demonstrated that B cells have a significant

325   phagocytic capacity, being able to phagocytose particles including bacteria [42-44]. Most

326   important has been the demonstration of the efficient capability of these cells to present

327   antigen from phagocytosed particles to CD4$^+$ T cells [42-44], acting as a bridge that link

328   innate with adaptive immunity. Therefore, considering the inferred high connection of

329   these phagocytosis phenotypes with gut microbiota, we could hypothesize that, as other

330   antigen-presenting cells such as dendritic cells or macrophages, the phagocytic

331   lymphocytes seem to be relevant to maintain immune tolerance to the normal gut

332   microbiota, being also relevant to control the abundance of opportunistic pathogens. B-

333   cells also produce secretory IgA, the most abundant secreted isotype in mammals and a

334   key element to maintain 'homeostatic immunity'[45]. Secretory IgA was among the most

335   connected traits in the network being positively associated with several taxa. Among them,

336   the genus *Blautia* is of particular interest due to its potential role modulating inflammatory

337   and metabolic diseases, with potential beneficial effects for the host [46]. Therefore,

338   similar to phagocytic lymphocytes, the interplay between secretory IgA levels and the

339   abundance of different taxa in our animals may regulate the ecological balance of

340   commensal bacteria and the development of Ig-A secreting cells. Neutrophils were also

341   positively correlated with gut microbiota profiles. A systemic immunomodulation of

342   neutrophils by intestinal microbiota has been demonstrated [47], and a crosstalk between

343   NEU and gut microbial composition has been also documented [48].   Our results

344   confirmed a positive association of *Oribacterium* abundance with the quantity of

345   neutrophils. *Oribacterium* genus belongs to the *Lachnospiraceae* family, and the

346   abundance of this genus increased in piglets after weaning [49]. Members of the genus

347   *Oribacterium* produce short-chain fatty acids such as acetate [50], which directly

348   influences immune system regulation [51], and can contribute to the health of the pig.

349   Among the most connected traits in our network we also found acute-phase protein

350   Hp, which based on the estimated microbiability appeared preferentially determined by

351   microbiota effects. The main function of Hp is to facilitate hemoglobin (Hb) clearance.

352   After the formation of stable Hp-Hb complexes, the macrophage receptor CD163

353   recognize them and the entire complex is removed from circulation by receptor-mediated

354   endocytosis [52]. Therefore, Hp favors the reduction of free iron concentrations in the

355   circulation and tissues [53]. Several bacterial pathogens such as *Staphylococcus*,

356   *Mycobacteria*, *Salmonella*, *Corynebacterium*, *Haemophilus*, among others, require iron for

357   growth, thus elaborating different acquisition strategies to uptake heme from the host,

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358   particularly from Hb [54-56]. The host immune system has developed antimicrobial
359   mechanisms, most related to innate pathways, to deplete iron availability for pathogens
360   [54]. Remarkably, our results indicate a relevant effect of the microbiota composition on
361   Hp levels which could also modulate the concentration of circulating free iron. We could
362   hypothesize that the symbiotic microbiota could also modulate the iron levels in these
363   animals through innate immunity mechanisms to prevent the development of different
364   opportunistic pathogens. Our results confirmed a positive association between serum
365   concentration of Hp and the relative abundance of *Megasphaera*, a member of the phylum
366   Firmicutes. According to this result, an increase in the abundance of *Megasphaera* has
367   been described in colon content and faeces of pigs fed with iron-deficient diet [57].
368   Interestingly, this genus was reported as a potential biomarker for immune-mediate
369   mechanism of protection from diarrhea [58] and positive correlated with luminal IgA
370   concentration in pigs [49].

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372   Finally, it is worth highlighting the negative association between the relative
373   abundance of *Desulfovibrio* and LEU and MON counts. *Desulfovibrio* is a sulfate-reducing
374   bacteria (SRB), which can promote the metabolism of sugars [59] and plays also a key role
375   in intestinal hydrogen and sulfur metabolism [60]. In pigs, *Desulfovibrio* plays a relevant
376   role during pig gut colonization [49] and was among the dominant genus in healthy pigs
377   compared with diarrhea-affected piglets [61]. In fact, in weaned piglets, a negative
378   correlation between *Desulfovibrio* and several inflammatory markers such as IL-1β, IL-2
379   and IL-6, have been observed [62], which would be in agreement with the negative
380   correlation observed between *Desulfovibrio* and LEU and MON counts in our piglets.

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382   Despite of an inventory of potential gut health biomarkers exists for pigs [63, 64],
383   our results propose new microbial candidates, and emphasize a polymicrobial nature of
384   the immunocompetence in pigs. Furthermore, in agreement with previous reports [65], our
385   results suggest that some immunity traits are influenced by specific microorganisms while
386   others are determined by interactions between members of the gut microbiome. We also
387   reveal the joint contribution of the host genome and the gut microbial ecosystem to the
388   phenotypic variance of immunity parameters and advice that ignoring microbiota effects
389   could generate an overestimation of genetic parameters. A better understanding of the host
390   and microbial contribution to immunocompetence will allow to develop holistic breeding

391 strategies to modulate these traits, as well as to improve animal health, robustness and
392 welfare.
393

Conclusions

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396 Estimates of heritability and microbiability exposed the joint contribution of both
397 the host genome and the gut microbial ecosystem to the phenotypic variance of immunity
398 parameters, and revealed that ignoring microbiota effects on phenotypes could generate an
399 upward bias in the estimation of genetic parameters. Results from the MWAS suggested a
400 polymicrobial nature of the immunocompetence in pigs and highlighted associations
401 between the compositions of pig gut microbiota and 15 of the analyzed immunity traits.
402 Overall, our findings establish several links between the gut microbiota and the immune
403 system in pigs, underscoring the importance of considering both sources of information,
404 host-genome and microbial level, for the genetic evaluation and the modulation of
405 immunocompetence in pigs.
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