

# Contents

<b>Acknowledgments</b> .....	<b>V</b>
<b>Contents</b> .....	<b>VII</b>
<b>List of Figures</b> .....	<b>XI</b>
<b>List of Tables</b> .....	<b>XIII</b>
<b>Summary</b> .....	<b>XIV</b>
<b>Resumen</b> .....	<b>XVI</b>
<b>Resum</b> .....	<b>XVIII</b>
<b>1. Literature review</b> .....	<b>1</b>
1.1. Intramuscular fat and its role in meat quality.....	1
1.2. Intramuscular fat metabolism .....	2
1.3. Strategies to improve IMF content.....	3
1.3.1. Divergent selection for IMF in rabbits.....	4
1.4. The <i>-omics</i> approach .....	6
1.4.1. The beginning of an era.....	6
1.4.2. Microbiome: the second genome of the host .....	7
1.4.3. Metabolome: the final expression before the phenotype .....	10
1.5. Analytical problems .....	11
1.5.1. Multivariate analyses .....	12
1.5.2. The compositional problem.....	12
1.6. References .....	14
<b>2. Aim of the Thesis</b> .....	<b>24</b>
<b>SECTION I: Analysis of the IMF deposition from the host perspective</b> .....	<b>27</b>
<b>3. Intramuscular fat selection in rabbits modifies the fatty acid composition of muscle and liver tissues</b> .....	<b>28</b>
3.1. Abstract .....	29
3.2. Background.....	29
3.3. Methods.....	30
3.3.1. Animals.....	30
3.3.2. Sampling .....	31
3.3.3. Statistical Analysis.....	33

3.4.	Results and discussion .....	34
3.4.1.	Direct and correlated response to selection after 10 generations.....	34
3.4.2.	Correlated response in the meat fatty acid content.....	35
3.4.3.	Correlated response in liver fat and plasma metabolites.....	37
3.4.4.	Correlated response in the liver fatty acid content .....	38
3.5.	Conclusions.....	40
3.6.	References.....	41
<b>4.</b>	<b>Plasma metabolomic profiling in two rabbit lines divergently selected for intramuscular fat content.....</b>	<b>44</b>
4.1.	Abstract.....	45
4.2.	Background.....	45
4.3.	Methods .....	46
4.3.1.	Animals .....	46
4.3.2.	Plasma metabolome .....	47
4.3.3.	Data processing.....	49
4.3.4.	Statistical analysis.....	49
4.4.	Results and discussion .....	52
4.4.1.	Lipids metabolism.....	54
4.4.2.	Amino acids metabolism .....	56
4.4.3.	Metabolic pathways related to the microbiome metabolism.....	57
4.4.4.	Other metabolic pathways affected by selection .....	58
4.5.	Conclusions.....	59
4.6.	References.....	59
4.7.	Supplementary information.....	63
<b>SECTION II: Analysis of the IMF deposition from the microbiome perspective..</b>		<b>64</b>
<b>5.</b>	<b>Divergent selection for intramuscular fat shapes the gut enterotypes .....</b>	<b>66</b>
5.1.	Abstract.....	67
5.2.	Background.....	67
5.3.	Methods .....	69
5.3.1.	Animals .....	69
5.3.2.	DNA extraction, library construction and sequencing.....	70
5.3.3.	Sequence bioinformatics analysis .....	70
5.3.4.	Computation of alpha diversity.....	71
5.3.5.	Microbiome data treatment.....	71
5.3.6.	Correlated response to selection on the microbiome composition .....	72

5.3.7.	Search of microbial biomarkers for IMF prediction.....	73
5.4.	Results and discussion.....	74
5.4.1.	Caecum microbiome composition in rabbits .....	74
5.4.2.	Divergent IMF-enterotypes as a response to selection .....	75
5.4.3.	Main microbial drivers of the two IMF-enterotypes .....	78
5.4.4.	Microbial biomarkers to predict host IMF genetic background.....	81
5.5.	Conclusions .....	82
5.6.	References .....	83
5.7.	Supplementary information .....	88
<b>6.</b>	<b>Comprehensive functional core microbiome comparison in genetically obese and lean hosts under the same environment .....</b>	<b>95</b>
6.1.	Abstract .....	96
6.2.	Background.....	96
6.3.	Methods.....	98
6.3.1.	Animals.....	98
6.3.2.	Lipid content measurement and caecal samples collection .....	99
6.3.3.	Microbial gene abundance measurements.....	99
6.3.4.	Statistical analysis.....	100
6.4.	Results and discussion.....	102
6.4.1.	Microbial metabolism of lipopolysaccharides, peptidoglycans, lipoproteins and mucin components .....	104
6.4.2.	Microbial energy production and conversion pathways .....	109
6.4.3.	Microbial metabolism of lipids.....	109
6.4.4.	Microbial metabolism and transport of amino acids .....	110
6.4.5.	Microbial antibiotic resistance.....	110
6.4.6.	Other microbial pathways linked to IMF genetics .....	111
6.4.7.	Different microbial mechanisms influence lipid deposition in the muscle and the main body depots.....	111
6.5.	Conclusions .....	112
6.6.	References .....	113
6.7.	Supplementary information .....	119
<b>7.</b>	<b>The caecum metabolome of two divergently selected lines reveals microbial biological mechanisms involved in intramuscular fat deposition.....</b>	<b>120</b>
7.1.	Abstract .....	121
7.2.	Background.....	121
7.3.	Methods.....	123

7.3.1.	Animals .....	123
7.3.2.	Cecum metabolome.....	123
7.3.3.	Data processing.....	124
7.3.4.	Statistical analysis and validation .....	125
7.3.5.	Biomarker selection .....	127
7.4.	Results .....	127
7.4.1.	Multivariate analysis .....	127
7.4.2.	Microbial metabolic pathways affected by selection.....	128
7.4.3.	Potential biomarkers.....	132
7.5.	Discussion.....	132
7.5.1.	Amino acids and peptides.....	132
7.5.2.	Lipids .....	134
7.5.3.	Nucleotides.....	135
7.5.4.	Potential biomarkers.....	136
7.6.	Conclusion .....	136
7.7.	References.....	137
7.8.	Supplementary information.....	141
<b>8.</b>	<b>General discussion.....</b>	<b>162</b>
8.1.	References.....	167
<b>9.</b>	<b>General conclusions .....</b>	<b>171</b>

# List of Figures

<b>Figure 4.1</b> –Cross model validation (CMV) diagram. ....	51
<b>Figure 4.2</b> –Adjustment parameters of the PLS models. Distribution of all the $Q^2$ values obtained from the PLS models adjusted during the cross-validation procedure with <b>(a)</b> true data, and with <b>(b)</b> permuted data. ....	53
<b>Figure 4.3</b> –Summary of the relevant metabolites obtained with the PLS and PLS-DA models. <b>(a)</b> Venn diagram of the metabolites selected using PLS-DA and PLS approaches; <b>(b)</b> classification of the 322 metabolites selected. ....	53
<b>Figure 4.4</b> –Lipids sub-pathways. Number of differentially abundant metabolites found for each lipids sub-pathway. ....	54
<b>Figure 4.5</b> –Amino acids sub-pathways. Number of differentially abundant metabolites found for each amino acids sub-pathway. ....	57
<b>Figure 5.1</b> – Divergent caecal enterotypes of high (H) and low (L) intramuscular fat (IMF) lines as a result of ten generations of selection for IMF. ....	77
<b>Figure 5.2</b> – Distribution of microbial biomarkers or balances for prediction of intramuscular fat (IMF) genetic background, by enterotypes and by IMF. ....	82
<b>Figure S5.1</b> – The first two components of a principal components analysis (PCA) of the composition of the caecal microbiome after additive log-ratio transformation at phylum <b>(a)</b> and genus <b>(b)</b> levels show the sequencer effect (FISABIO or Sist. Genómicos Laboratories) before and after correction. ....	89
<b>Figure S5.2</b> – Selection of the reference variable (in red) for the additive log-ratio (alr) transformation at <b>(a)</b> genus level ( <i>Eubacterium</i> ) and <b>(b)</b> phylum level (Fibrobacteres). ....	89
<b>Figure S5.3</b> – Divergent caecal enterotypes at phylum level of high (H) and low (L) intramuscular fat (IMF) lines as a result of ten generations of selection for IMF. ....	90
<b>Figure S5.4</b> – Pearson correlation between the additive log-ratio (alr) transformed abundances of <b>(a)</b> 51 genera or <b>(b)</b> 10 phyla distinguishing between the two enterotypes of high and low intramuscular fat content. Data were adjusted for sequencing and sex effects before calculating correlations. ....	91
<b>Figure 6.1</b> – Comprehensive identification of caecal microbial gene (MG) additive log-ratio ( <i>alr</i> ) transformed abundances evidencing a correlated response to selection for intramuscular fat content. ....	106
<b>Figure 6.2</b> – Differences between obese and lean lines in microbial gene (MG) additive log-ratio ( <i>alr</i> ) transformed abundances presenting a correlated response to selection for intramuscular fat content. ....	107
<b>Figure 6.3</b> – Top 10 microbial taxa highly enriched with unique proteins classified within KEGG Orthologue groups (or microbial genes) presenting a correlated response to selection for intramuscular fat content. ....	108
<b>Figure 7.1</b> – Venn diagram of the metabolites selected using PLS-DA, PLS-H, and PLS-L approaches. ....	128

**Figure 7.2** – Classification of the relevant metabolites selected in the PLS-DA, PLS-H and PLS-L models according to their chemical nature..... 129

**Figure 7.3** – Classification of the relevant metabolites according to the metabolic pathways in which they are involved selected in the **(a)** PLS-DA, **(b)** PLS-L and **(c)** PLS-H models. .... 131

**Figure 7.4** – Distribution of the balance (*itr*) according to the genetic line..... 132

# List of Tables

<b>Table 3.1.</b> Descriptive statistics and differences between lines of meat and carcass quality traits.....	34
<b>Table 3.2.</b> Descriptive statistics and differences between lines of the meat fatty acid profile. ....	36
<b>Table 3.3.</b> Descriptive statistics and differences between lines of liver traits and plasma metabolites related to liver metabolism. ....	38
<b>Table 3.4.</b> Descriptive statistics and differences between lines of the liver fatty acid content. ....	39
<b>Table 4.1.</b> Adjustment parameters obtained from the predictions of the PLS-DA model during the CMV procedure. ....	52
<b>Table S5.1.</b> Differences between genetic IMF-enterotypes in additive log ratio transformed abundance of selected phyla or genera presenting a correlated response to selection.....	92
<b>Table S7.1.</b> Results from the statistical analysis of the 142 relevant metabolites for the discrimination between lines (PLS-DA).....	142
<b>Table S7.2.</b> Results from the statistical analysis of the 107 relevant metabolites in the L line (PLS-L).....	149
<b>Table S7.3.</b> Results from the statistical analysis of the 156 relevant metabolites in the L line (PLS-H).....	154