FEED-A-GENE

Adapting the feed, the animal and the feeding techniques to improve the efficiency and sustainability of monogastric livestock production systems

Deliverable D5.2

New traits to select for feed efficiency

Due date of deliverable: M48

Actual submission date: M48

Start date of the project: March 1st, 2015   **Duration:** 60 months

Organisation name of lead contractor: INRA

Revision: V5

Dissemination level	
Public - PU	**X**
Confidential, only for members of the consortium (including Commission Services) - CO	
Classified, as referred to in Commission Decision 2001/844/EC - Cl	

Table of contents

1. Summary

Objectives. Selection in monogastric species is applied to pure lines in selection farms, while commercial animals are crossbreds raised in diverse conditions. Improving feed efficiency in these populations is a key to enhance the productivity and limit the environmental footprint of livestock production. However, recording feed efficiency is costly in most species because it requires measuring feed intake, and this trait is affected by genotype by environment interactions (GxE) that makes it a different trait in selection and commercial populations. Major gains in genetic progress could thus be achieved if more animals had records for feed intake or feed efficiency, and if these measurements could be obtained from any farm. Identifying new traits for selection of feed efficiency is thus crucial to improve the prediction accuracy of breeding values in livestock populations.

Depending on the species, measuring feed efficiency on-farm is a difficult issue: individual feeders for pigs have been available on-farm for long. They are costly to acquire and maintain, but at least they provide reference measurements in most populations. Poultry and rabbits still rely on measurements made in individual cages. This type of measurement is not representative for the performance of animals raised in groups, and is questioned in terms of welfare. Thus, our objectives were:

1. To test direct measures of feed intake and feed efficiency for genetic designs after the development of electronic feeders in WP2 for rabbits
2. To evaluate measures of components of feed efficiency (i.e., digestibility, activity and behaviour, robustness), which could be used to select more efficiently individuals dedicated to different breeding conditions when GxE is large. A major effort was undertaken to understand the contribution of the gut microbiota to feed efficiency and its potential as a criterion for selection, which is reported in a separate deliverable (D5.1)
3. To identify biological markers of feed efficiency and their components that could be measured on a large number of individuals at a moderate cost, potentially on production farms, so that selection accuracy for production conditions could be improved.

Rationale. To respond to these objectives, data and technologies from WP2 (i.e., new traits for feed efficiency) and new trials were combined to evaluate feed efficiency under a wide range of conditions, including different feed resources, different breeding systems, and different physiological stages of the animal. Indeed, reproduction has long been ignored when considering feed efficiency issues, while it has a major impact on management of body reserves and on female longevity. To ensure that the proposed solutions would not have a negative impact on other production traits of interest, indicators of robustness and product quality were recorded.

Classical genetic methodologies have been applied, either by comparing genetic lines selected for the trait of interest for multiple generations so that the genetic difference between animals for this trait has been established (i.e., the residual feed intake (RFI) lines in pigs, rabbits and layers) and the correlated response on other traits or indicators can be measured, or by measuring the traits in large cohorts of conventional populations (i.e., Large White and crossbred pigs, Caldes rabbits) or alternative lines (i.e., Duroc and Iberian pigs). When using selected lines, the genetic aspect of the response can be observed directly by comparing the mean line responses, whereas in large pedigreed cohorts,

animal linear mixed models were applied to estimate genetic variances and heritabilities. To detect genomic markers associated with the traits of interest, the same animal linear mixed models can be used, including a SNP effect in iterative tests along the genome. Because feed intake is sometimes not available at the individual level, an original model based on a bivariate development of the same linear model was tested in rabbits to detect associated SNP with traits recorded in groups. Finally, multivariate models dedicated to the simultaneous analysis of large number of variables were used in transcriptomic studies to account for the number of repeated tests and the specificity of these data.

In a first set of analyses, new traits could be validated as heritable in the tested populations. For growing animals, these traits include measures of components of feed efficiency, such as feed intake records measured by automatic feeders in rabbits and digestibility indicators measured in group-housed pigs (i.e., through direct NIRS prediction) and in poultry (i.e., though indirect prediction via serum absorbance). The digestive efficiency in pigs was tested with a conventional and with a high dietary fibre diet, and the analysis showed that within the range of digestibility values explored, no strong genotype-by-diet interaction was observed for digestibility. Although digestive efficiency was strongly correlated with feed efficiency, some moderate adverse correlations were estimated with other production traits (i.e., carcass yield and meat quality traits). In reproductive females, using individual feed intake data from gestating sows appeared to be difficult in genetic studies, especially in relation with different management systems of the sows. In one study, a reasonable variability seemed to be available (Large White pigs in a French farm) whereas very little variability was observed in a second dataset for this period (Duroc pigs in a Spanish farm). Larger and more diverse datasets would be necessary to explore how and when management limits the expression of genetic variability in this period, so a more complete analysis could be envisaged. However in Duroc sows, records of lactation traits led to estimations of the genetic variability of feed intake and feed efficiency during this period. Despite a limited number of feed intake records, the estimates were high enough to envisage selection on these traits with a limited additional phenotyping effort. Additionally and for the first time, an estimation of the genetic variability of feed intake and feed efficiency during lactation in Iberian sows was provided. Finally, some components of feed efficiency, such as behaviour, activity, welfare, and robustness were also considered, as they can positively or negatively contribute to feed efficiency. Because direct measures of activity were not available, indirect indicators were considered. The first type of indicators focused on traces of interactions on the animal's body. However, only few traits had high enough heritabilities and correlation with feed efficiency to be used to refine the accuracy of actual estimations. The second type of indicators were derived from automatic feeder records of animal activity: feeding behaviour traits were shown to be heritable (e.g., number of visits and feeding rate) and they had some genetic correlations with production traits. In the two datasets explored, correlations were higher with feed intake than with feed efficiency. In addition, feeding patterns could be used, either empirically or via a ranking approach, to propose indicators of the animal hierarchy in the pens. Interestingly, the more dominant animals are not necessarily more efficient. This novel aspect needs further analysis to be used in selection. Finally, welfare indicators were measured in the blood and in pig hair. Blood cell counts seemed to have promising genetic correlations with feed efficiency traits, which need to be explored further. Robustness indicators were tested in divergent lines, following the hypothesis that more efficient individuals would be less robust. The hypothesis was not sustained by the experiment, which was consolidated by a mirror experiment in which divergent animals for robustness were compared for their feed efficiency, with no deleterious effect of selection for robustness on the production traits.

In a second set of analyses, biological markers of feed efficiency at the genomic and the transcriptomic levels were identified. A first strategy, based on the sequencing of divergent layer lines, allowed the identification of 145 SNP differing between lines and candidates to be associated with feed efficiency. In rabbits, first analyses of a recently available SNP chip were run in two different populations. Four to five genomic regions were associated to the trait variability in each population, with no common region. In broilers, the genomic associations with digestibility traits indicated 12 significant SNPs. A few genes were identified as potential candidates for these regions, which needs further validation. Finally, expression studies were run between divergent lines to identify the biological pathways involved in the line differences in response to different treatments, as well as to identify biomarkers in layers and in pigs. In layers, the animals were slaughtered and multiple tissues with a potential impact on feed efficiency were sampled. In pigs, serial measurements were applied to blood samples. In both cases, some genes were identified as responsible for the differences between the lines. However, the genes were partly diet- or time-dependent and the way they contribute to the base difference versus the treatment difference needs to be explored further to propose biomarkers dedicated to specific situations.

Teams involved: INRA, IRTA, TOPIGS NORSVIN, IFIP

Species and production systems considered: pigs, rabbits, broilers, and layers were considered, including conventional populations and alternative populations (i.e., Iberian pigs)

2. Introduction

Identifying new traits for selection for feed efficiency is crucial to improve selection accuracy in livestock populations. Depending on the species, measuring feed efficiency on-farm is a difficult issue: individual feeders for pigs have been available on-farm for long, whereas poultry and rabbits still rely on measurements in individual cages. This type of measurement is not completely representative for the performance of animals raised in groups, and is questioned in terms of welfare. From a breeder's point of view, accurate prediction of feed efficiency of crossbred animals in production environments is another issue, as this trait is influenced by GxE interactions. Thus, our objectives were:

1. To test direct measures of feed intake and feed efficiency for genetic designs using the electronic feeders developed in WP2 for rabbits and broilers
2. To evaluate measures of the components of feed efficiency (e.g., digestibility, activity and behaviour, and robustness), which could be used to select more efficient individuals on these components if GxE is important.
   A major effort was undertaken to understand the contribution of the gut microbiota to feed efficiency and its potential for selection, which is reported in a separate deliverable (D5.1)
3. To identify biological markers of feed efficiency and their components that could be measured on a large number of individuals at a moderate cost, potentially on production farms, so that selection accuracy in production conditions could be improved.

To respond to these objectives, data and technologies from WP2 (i.e., new traits for feed efficiency), and new trials were combined to evaluate feed efficiency under a wide range of conditions, including different feed resources, different breeding systems, and different physiological stages of animals. To ensure that the proposed solutions would not have a negative impact on other production traits of interest, indicators of robustness and product quality were also recorded.

3. Results

3.1 Genetic variance of new feed efficiency traits

3.1.1 Feed efficiency

➤ Estimates of heritability of feed intake in rabbits

Experimental design. Using the data of 268 rabbits documented in deliverable D2.3 for the design of the individual feeders in rabbits, individual average daily feed intake (ADFI) and average daily gain (ADG) recorded in 2018 were used to obtain first estimates of heritabilities of these two traits, considering only one generation of relationships between animals. Table 1 presents the raw statistics for these traits by batch, together with the phenotypic correlation between ADFI and ADG within batch

Table 1. Raw statistics for average daily feed intake (ADFI) and average daily gain (ADG) recorded in three batches in 2018.

Batch (AI date)	N	ADFI* (g/d)	ADG (g/d)	FCR*	Correlation (ADFI-ADG)
1	90	139.8	47.7	2.9	0.51
2	106	129.3	49.5	2.6	0.35
3	72	143.3	49.8	2.9	0.31

* ADFI and FCR were computed using only valid visits to the feeder.

Results and discussion. Table 2 shows the variance components and heritability estimates of the traits, estimated using an animal model, using litter and cage as random effects, in addition to the additive genetic random effect. The EM-REML heritability estimate for ADFI was 0.29.

Table 2. Variance components and heritability estimates for average daily feed intake (ADFI) and average daily gain (ADG).

Source	ADG (g/d)	ADFI (g/d)
Cage	23.55	33.65
Litter	0.25	2.20
Additive	39.35	95.91
Residual	20.46	196.1
Phenotypic	83.61	327.86
h^2	0.47	0.29

A breeding program directly considering both ADFI and ADG, or alternatively considering an index reflecting the individual efficiency of the animals (e.g., feed conversion ratio (FCR) or residual feed intake (RFI)) can thus be envisaged. As indicated in deliverable D2.3, although validated individual feed intake records seem to show a downward bias of the actual feed intake, we expect these records to be accurate enough to estimate accurate breeding values and rank individuals for their potential for feeding traits in breeding programs to improve feed efficiency (i.e., reduce feed intake while increasing growth).

➤ Genetic of digestive efficiency

Most commercial breeds in pig and poultry are selected for feed efficiency using high-quality feeds. These feeds are easy to digest, and provide sufficient energy and all required nutrients so that animals

can express their growth potential. If including alternative feedstuffs (e.g., by-products from the agrofood industry) in feeds may be a solution to reduce costs and enhance the use of locally grown crops, it may also alter the ability of animals to convert efficiently feed into muscle, especially when feed ingredients are difficult to digest and the nutrients supply becomes limiting. Hence, digestive efficiency can be novel trait to exploit for further improving the feed efficiency in growing animals. In broilers, variability in digestive efficiency among animals have been proven to exist when they are fed alternative feedstuff (e.g., a challenge diet), part of which can be explained by genetics (Grasteau *et al*., 2004, 2010). A near-infrared spectrophotometry (NIRS) technique has been developed to assess digestive efficiency in chickens, which offers a great potential to improve the digestive efficiency by measuring this trait in large numbers of animals. However, this technique still requires the total collection of faeces and thus to rear animals in individual cages, which raises welfare concerns. It also generates genotype-by-environment interactions, as animals are normally reared in groups and on the floor. Alternative indicators of digestive efficiency that could be measured in group-housed animals on the floor were therefore evaluated, to measure larger cohorts of animals in normal rearing conditions.

In pigs, preliminary studies suggest that a genetic variation in digestive efficiency might exist (Noblet *et al*., 2013). To test this hypothesis, a methodology was developed in WP2 (deliverable D2.5) allowing individual prediction of nutrient digestibility based on NIRS analysis of faeces collected at a single point in time in animals kept in groups. It alleviates many of the constraints imposed by the gold standard method to measure digestibility (i.e., the use of metabolic cages to collect all faeces of pigs during a period of time, followed by extensive laboratory analyses to determine the nutrient and energy contents in both the feed and the faeces). This new NIRS-based method was tested as a high-throughput phenotyping method for digestibility on large cohorts of pigs reared in selection farms, comparing responses to a conventional and a fibrous diet.

o   Biomarkers of digestive efficiency in broilers

Experimental design. The use of metabolome data to predict digestive efficiency has been evaluated in WP2 (task 2.5). Models built on a limited number of animals (N=60) showed that the most predictive criterion was absorbance of blood serum at 492 nm. To validate this trait for breeding, serum was collected from 417 animals of a medium-growing meat-type broiler line. Blood was sampled as 3 weeks of age, age at which the link between serum absorbance and digestive efficiency was shown to be maximum. The absorption spectra were acquired every 2 nm between 342 nm and 572 nm using an Infinite M200 spectrophotometer. Digestive efficiency was assessed by measuring the apparent metabolisable energy (AMEn). Genetic analyses was performed using the VCE6.0 software (Neumaier and Groeneveld, 1998; Groeneveld *et al*., 2010), using an animal model including fixed effects of hatch, rearing cell, sex, and plate for analysis of serum absorbance.

Results. Figure 1 shows the heritability of absorbance of blood serum between 342 and 572 nm, the genetic correlation between AMEn and absorbance, and the expected response of AMEn to selection of serum colour for each wavelength. Taking into account these genetic parameters, the most interesting criterion was serum colour at 492 nm, with a heritability estimated at 0.31±0.09, and a genetic correlation with AMEn estimated at 0.84±0.28. The genetic correlation between serum absorbance and body weight was not significantly different from 0 between 382 and 522 nm (0.29±0.27 at 492 nm).
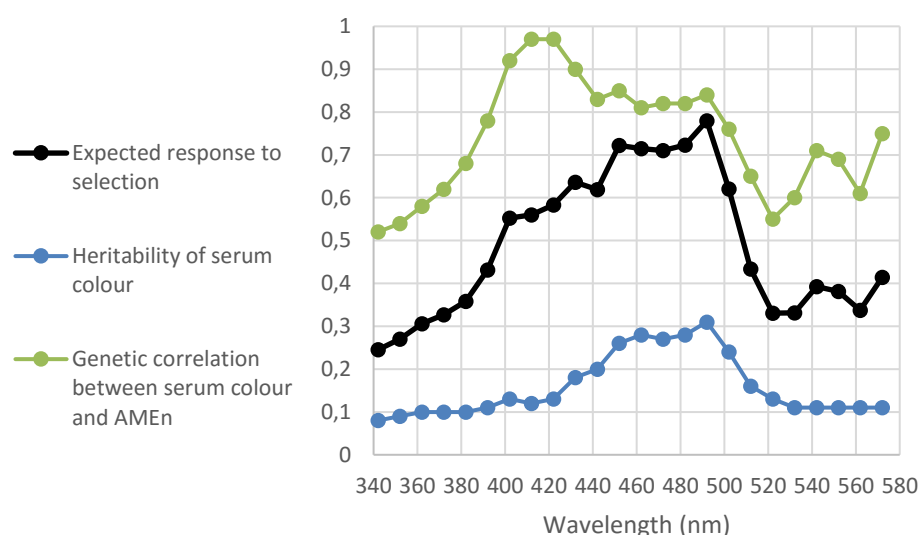
Figure 1. Genetic parameters of serum colour and expected response of apparent metabolisable energy (AMEn) to selection of serum colour.

Main discussion points and conclusion. Taking into account these parameters, the most interesting biomarker to be used to select AMEn is serum absorbance at 492 nm as, with a heritability estimated at 0.31±0.09 and a genetic correlation with AMEn estimated at 0.84±0.28, it provides the highest expected response to selection (Figure 1). These results have been submitted for publication (Mignon-Grasteau *et al.*, 2018). The next steps will be to validate this criterion on a larger number of genotypes and diets.

o   Genetic determinism of digestive efficiency in pigs

Experimental design. A sample collection was set up to estimate the genetic parameters of digestive efficiency traits in growing pigs and quantify their genetic relationships with feed efficiency and other selected traits (i.e., carcass composition and meat quality). Two different diets were used to evaluate the genotype-by-diet interaction for these measurements: a conventional diet (CO) and an alternative diet (HF) that had a higher crude fibre content due to the addition of various fibrous by-products (i.e., wheat bran, soybean hulls, and dehydrated sugar beet pulp). Both diets had a composition close to those used in WP2 (deliverable D2.5, feed formula in Annex 1), which describes the development of the methodology to estimate digestive efficiency. The trial was carried out at the France Génétique Porc test station (Le Rheu, France) as part of the national breeding test of the Large White population. In total, 1,598 purebred Large White animals entered the station between February 2017 and July 2018 in 29 successive batches of about 56 animals (i.e., 4 pens of 14 animals). Couples of full sibs (entire males) from ten different breeding herds were tested with the two diets. Individuals were grouped at weaning, shipped to the test station to receive the same feed until 9 weeks of age (i.e., until the start of the growing phase). Then, full sibs were separated and each pen received either the CO or the HF diet until the end of the test (120 kg). Individual feed intake and body weight were recorded automatically with electronic feeders and automatic scales. Those records allowed calculating individual average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) from 35 kg to 120 kg. Faecal samples were collected from every pig at an average weight of 65 kg, and were immediately frozen at -20°C. All animals were slaughtered when they reached 120 kg live weight. Carcass and meat quality traits (i.e., lean meat content, carcass yield, ultimate pH of the ham) were

recorded. Faecal samples were processed following the protocol provided in deliverable D2.5 and analysed using a near-infrared spectrophotometer. For each animal, individual digestibility coefficients for energy, organic matter, and nitrogen were predicted.

Results. Consistently with results obtained in deliverable D2.5, the digestibility coefficients of energy and nitrogen were reduced by 5 to 6 points with the HF diet compared to the CO diet, meaning that animals digested less energy and nitrogen when more dietary fibres were provided (Figure 2). However, the variability of digestibility coefficients was similar for both diets. Within each diet, digestibility coefficients of energy, nitrogen, and organic matter were highly correlated (>0.80), suggesting that animals that digested best energy were also the best ones at digesting nitrogen and organic matter.
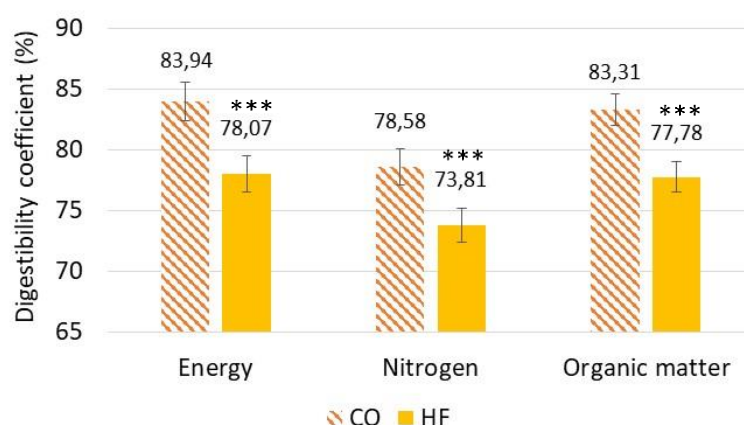

Figure 2. Mean digestibility coefficients of energy, nitrogen and organic matter estimated for pigs fed the conventional (CO) and high-fibre (HF) diet (***: P<0.01).

The HF diet had a clear impact on the mean of all traits, except for meat quality. The HF diet also affected the variability of traits related to feed efficiency (i.e., ADFI, FCR, and digestibility coefficients). In brief, animals fed the HF diet had on average a significantly lower growth rate (-54 g/day) and a higher ADFI (+157 g/day), resulting in a decreased FCR (+0.26 kg feed/kg live weight). In addition, animals fed the HF diet were leaner (LMC: +1.72%), potentially in relation with the effect of dietary fibre on the feed intake capacity of the animals, and had a lower carcass yield (-1.48%). This is in line with earlier studies in relation with increased intestinal transit rate resulting in development of the digestive tract (Montagne *et al.*, 2014).

Heritabilities

Traits measured in pigs fed the CO and the HF diets were analysed as different traits to estimate the genetic correlations between traits recorded on different diets.

Digestive efficiency was moderately to highly heritable for energy, nitrogen, and organic matter, meaning that some families of pigs digest feed more efficiently than do others (Table 3). Digestive efficiency coefficients were more heritable in the HF feed, as reported in broilers. For all other traits, estimated heritabilities were consistent with previously published estimates for both CO and HF diets: i.e. moderate to high values for growth, feed efficiency, and carcass traits and low to moderate values for meat quality traits.

Table 3. Heritability estimated for digestibility coefficients for both conventional (CO) and high-fibre (HF) diets (estimated standard errors in parentheses).

Digestibility coefficient	Heritability – CO diet	Heritability – HF diet
Energy	0.41 (0.14)	0.62 (0.17)
Organic matter	0.43 (0.14)	0.64 (0.17)
Nitrogen	0.50 (0.15)	0.70 (0.17)

Genetic correlations estimated between digestibility coefficients of energy, nitrogen, and organic matter, and other feed efficiency and carcass traits are given in Table 4 for the CO diet and in Table 5 for the HF diet. With both diets, digestibility coefficients of energy, nitrogen, and organic matter had negative (i.e., unfavourable) genetic correlations with ADG. On the contrary, genetic correlations between digestibility coefficients of energy, nitrogen, and organic matter were high and negative (i.e., favourable) with both ADFI and FCR. Hence, whatever the diet considered, families of pigs able to digest efficiently will genetically tend to have lower ADFI and FCR, but also a lower ADG. Genetic correlations between digestibility coefficients and lean meat content were different depending on the diet. They were moderate and favourable when using the conventional diet, and close to 0 when using the HF diet. With both diets, digestibility coefficients were slightly negatively (i.e., unfavourably) correlated with carcass yield. Finally, genetic correlations estimated between digestibility coefficients and meat quality traits were moderate and unfavourable. However, given the low heritability of meat quality traits, the estimation accuracy of those parameters was very low. More data would be needed to refine these estimations of genetic correlations between meat quality trait and digestive efficiency.

Table 4. Genetic correlations estimated between digestibility coefficients, feed efficiency, and carcass traits for pigs fed the conventional diet (estimated standard errors in parentheses).

Digestibility coefficient	ADG	ADFI	FCR	LMC	CY	uPH
Energy	-0.52 (0.23)	-0.83 (0.17)	-0.75 (0.27)	0.26 (0.11)	-0.24 (0.11)	NE
Organic matter	-0.53 (0.23)	-0.83 (0.16)	-0.74 (0.27)	0.29 (0.11)	-0.21 (0.11)	NE
Nitrogen	-0.56 (0.22)	-0.60 (0.27)	-0.50 (0.25)	0.18 (0.10)	-0.18 (0.10)	-0.42 (0.40)

ADG: average daily gain; ADFI: daily feed intake; FCR: feed conversion ratio; LMC: lean meat content; CY: carcass yield; uPH: ultimate pH of the ham; NE: not estimated.

Table 5. Genetic correlations estimated between digestibility coefficients, feed efficiency, and carcass traits for pigs fed the high-fibre diet (estimated standard errors in parentheses).

Digestibility coefficient	ADG	ADFI	FCR	LMC	CY	uPH
Energy	-0.65 (0.24)	-0.63 (0.23)	-0.62 (0.23)	0.03 (0.09)	-0.12 (0.08)	-0.45 (0.39)
Organic matter	-0.58 (0.24)	-0.59 (0.16)	-0.33 (0.40)	0.12 (0.09)	-0.11 (0.08)	-0.44 (0.39)
Nitrogen	-0.53(0.23)	-0.57 (0.22)	-0.51 (0.25)	0.16 (0.08)	-0.15 (0.07)	-0.51 (0.36)

ADG: average daily gain; ADFI: daily feed intake; FCR: feed conversion ratio; LMC: lean meat content; CY: carcass yield; uPH: ultimate pH of the ham.

To evaluate how pig performance would evolve due to selection on digestibility coefficients, the phenotypic differences between the CO progeny of the 25% highest and 25% lowest sires ranked on their average breeding value was calculated for digestibility coefficients of energy and nitrogen (Table 6). Phenotypes of progeny were pre-corrected for usual fixed effects. The phenotypic differences between groups were standardised by the phenotypic standard deviation (σ_p) of the traits to make them comparable between traits. The breeding value differences between the two groups of sires was

1.5 to 1.6 genetic standard deviations for digestibility coefficients of nitrogen and energy, respectively, which corresponds to a difference of digestibility of about 2.8% for nitrogen and 3% for energy. At their progeny level, as expected, a marked phenotypic difference (around 80% σ_p) was observed for digestibility coefficients between the two groups of progeny. The progeny of the 25% best sires were more feed-efficient, especially due to lower feed intakes (-61% σ_p), though their ADG was reduced to a lower proportion (-27% σ_p). The progeny of the 25% highest sires were slightly leaner than the other group (+16% σ_p), and they also had a lower carcass yield (-12% σ_p) and ultimate pH (-16% σ_p). These results confirm that selecting pigs for digestive efficiency should improve feed efficiency traits. However, carcass yield and meat quality traits should also be accounted for in breeding objectives to avoid altering these traits.

Table 6. Phenotypic differences between progeny groups of the 25% highest and 25% lowest sires ranked on their average breeding value for digestibility of energy and nitrogen.

Trait[1]	Phenotypic difference between groups of progeny	Difference standardised by phenotypic s.d.
ADG, g/d	-20.78	-27%
FCR, kg/kg	-0.07	-44%
ADFI , g/d	-128.6	-61%
Energy digestibility, %	1.96	+83%
Nitrogen digestibility, %	2.20	+77%
Organic matter digestibility, %	1.81	+83%
Lean meat content, %	0.36	+16%
Carcass yield, %	-0.15	-12%
Ultimate pH (ham)	-0.028	-16%

[1]ADG: average daily gain; FCR: feed conversion ratio; ADFI: daily feed intake.

Conclusions. Following methodological developments in WP2, the genetic determinism of digestive efficiency was characterised for the first time in growing pigs. The digestive efficiency of energy, organic matter, and nitrogen are heritable, meaning that there are opportunities to increase this(these) trait(s) by selection. High favourable genetic correlations were estimated with feed efficiency traits whatever the diet. Small to moderate adverse genetic relationships were identified with carcass yield and meat quality traits. The measure can be used routinely in breeding schemes of commercial pig breeds at a very moderate cost. It is possible to breed animals able to digest efficiently different feedstuffs, especially the more fibrous ones, to increase their robustness to variable feed composition.

After this proof of concept, the sensitivity of the genetic parameters to the feed and sampling conditions should be examined. In addition, the economic impact of feeding alternative feedstuffs can be evaluated from our results, to optimize the overall efficiency of a production system.

➢ Reproductive efficiency

No clear measurements of feed efficiency during the reproduction lifetime of the females have been documented. The main factor limiting progresses in this domain is the absence of routine measurements of feed intake, body weight, and indicators of the body composition of the females and the litter in most production systems. However, some data during lactation have been recorded in conventional systems, and measures of single lactation efficiency have been proposed (Bergsma *et al.*,

2008; Gilbert *et al*., 2012). The difficulty of obtaining individual feeding data in group-housed sows during gestation, and developing models to extract genetic variances from these data is a dimension that we dealt with here. It is common practice to apply a feed restriction to gestating sows according to parity and backfat thickness at the beginning of gestation, which might limit the opportunities to identify variability of genetic origin. When automatic feeders are available, the evaluation of the sow (feeding) behaviour could provide an insight in the biology of their reproductive efficiency.

- o Recording feed intake in group-housed gestating sows – variability and relation with reproduction traits in conventional systems

Experimental design. Large White sows raised in the Genesi INRA experimental facility (France) were considered for this study. Each pen with a capacity for 50 sows was equipped with an automatic feeder, and sows were identified individually with an RFID ear-tag. A feed restriction depending on backfat thickness and parity was applied based on a backfat measurement at beginning of gestation. This feeding plan was adjusted 30 d after artificial insemination based on the body condition of the sow. In the last third of lactation, all sows had their daily ration increased by 500 g. Data were available until 105 days of gestation, when the sows were moved to the lactation pens. The sow parity varied from 1 to 6, including a total of 375 gestation events. Feeders had doors at the entrance so that sows were not disturbed by other sows when eating. Sows had access to the feeder without restriction but the feeding trough closed when they had their total ration for the day, creating non-feeding visits. Data editing and analysis were performed with the R software. Models for feeding data included fixed effects such as backfat thickness at the start of the period and type of feeding ration provided to the sow for the day. Only sows with complete records were used in the analysis.

Results. Daily data showed that sows with more visits at the feeder spent the shortest total time at the feeder each day of gestation (phenotypic correlation r_p=-0.66±0.04) and had a lower feed intake at each visit (r_p=-0.59±0.05); their feeding rate per visit did not differ from that of sows having fewer visits at the feeder each day. The phenotypic correlations between feeding activity during gestation and lactation performance are shown in Table 7. The total number of visits at the feeder during the entire gestation period was positively correlated to the number of piglets weaned in the subsequent lactation, but was independent of litter size, litter weight, and sow body weight loss in the subsequent lactation. Positive and favourable correlations were obtained for the time spent at the feeder at each visit and the mean time spent at the feeder per visit in gestation with litter performance, except a negative correlation for the time spent at the feeder at each visit with the number of piglets weaned. Sows that consumed more feed per visit during gestation produced larger and heavier litters both at farrowing and at weaning, and lost more body weight to sustain the next lactation. Inversely, feed intake per visit and average time spent at the feeder per visit were negatively correlated with the number of piglets weaned. The feeding rate during gestation was negatively correlated with litter size and litter weight at weaning.

Table 7. Phenotypic correlations between feeding traits in gestation and lactation performance in a French Large White population (standard errors in parentheses).

Trait	NBA	NBT	LWB	NBW	LWW	SWD
NV_G	-0.028 (0.058)	-0.028 (0.058)	0.003 (0.058)	0.382 (0.053)	0.089 (0.058)	0.085 (0.058)
FI_G	0.287 (0.055)	0.287 (0.055)	0.523 (0.049)	0.172 (0.057)	0.388 (0.053)	0.597 (0.047)
FI_V	0.272 (0.055)	0.272 (0.055)	0.419 (0.052)	-0.168 (0.057)	0.203 (0.057)	0.383 (0.054)
TF_G	0.299 (0.055)	0.299 (0.055)	0.539 (0.048)	0.137 (0.057)	0.437 (0.052)	0.524 (0.05)
TF_V	0.252 (0.056)	0.252 (0.056)	0.389 (0.053)	-0.242 (0.056)	0.209 (0.056)	0.283 (0.056)
FR_G	-0.15 (0.057)	-0.15 (0.057)	-0.046 (0.057)	-0.218 (0.056)	-0.145 (0.057)	0.029 (0.058)

NBA: number of piglets born alive; NBT: number of piglets born in total; LWB: litter weight at birth; NBW: number of piglets weaned; LWW: litter weight at weaning; SWD: sow body weight difference between beginning and end of lactation; NV: number of visits; FI: feed intake; TF: time spent at the feeder; FR: feeding rate; _G: in gestation; _V: per visit.

Conclusions. During gestation, sows mainly had one feeding visit per day, so that visits can be used as elementary records. At the phenotypic level, some lactation performance traits were moderately associated with the feeding activity during gestation. Sows having more visits at the feeder (i.e., those with a higher activity at the feeder during gestation), were capable of weaning more piglets in the subsequent lactation. No incidence of performing more visits on litter size and litter weight was detected. Sows eating at lower rate in gestation appear to have better performance in lactation. Given the variability and correlations observed in this pilot study, the genetic relationships will now be investigated on a larger data set from the same population to evaluate if the detected correlations have a genetic basis or reflect the sow management.

> o Feed intake in Duroc sows during gestation and lactation, relationship with lactation efficiency

Experimental design. Data came from a Duroc population selected for prolificacy and backfat thickness at the end of the fattening period. They corresponded to two parities from 677 sows recorded from May 2015 to May 2016, distributed in 25 batches. During gestation, sows were housed in groups and were given once a day 2.16 kg of a standard diet containing 8.73 MJ of net energy, a minimum of 125 g of crude protein, 70 g crude fibre, and 6.6 g of total lysine per kg feed. About a week before parturition, sows were transferred to farrowing pens. Feed intake was limited to a maximum of 3 kg before farrowing. During lactation, sows were fed twice a day a standard feed containing 9.73 MJ of net energy, 166 g of crude protein, 9 g of total lysine, and a minimum of 49.1 g of crude fibre per kg feed. The feed supply was determined from the sow's feed intake during the previous day: it was increased when the sow finished her ration the day before, and was kept constant or reduced otherwise. The minimum and maximum amount of feed supplied daily were 2.22 and 9.62 kg/d, respectively. Daily patterns of feed intake are shown in Figure 3.

Automatic feeders were available in this farm only for group-housed sows from day 40 to 105 of gestation. Data recorded between 105 days of gestation and farrowing were eliminated to limit the high variability in feed intake during the pre-parturition time. A study of the best manual recording

Feed-a-Gene

pattern of feed intake for the other periods (early gestation and lactation) was first run to estimate the genetic parameters of feed intake during gestation and lactation, and their relationship with prolificacy traits. This preliminary evaluation indicated that feed intake during early gestation (i.e., until approximately 40 days of gestation) and during the lactation period could be manually recorded once or twice per week with no decrease in estimation accuracies. For missing daily records, daily feed intake was predicted using a 3rd order Legendre Polynomial function. Missing feed intake records during late gestation were predicted using a 6th order Legendre Polynomial function. Daily feed intake was calculated for early gestation (from day 1 to day 40 of gestation, FI_{1-40}), late gestation (from day 41 to day 105 of gestation, FI_{41-105}), and lactation (FI_{lac}). Feed intake during late gestation was divided into FI_{41-80} (from day 41 to day 80 of gestation), and FI_{81-105} (from day 81 to day 105 of gestation). FI_{1-40} and FI_{81-105} were highly variable, whereas FI_{41-80} had very low variability (Figure 3).
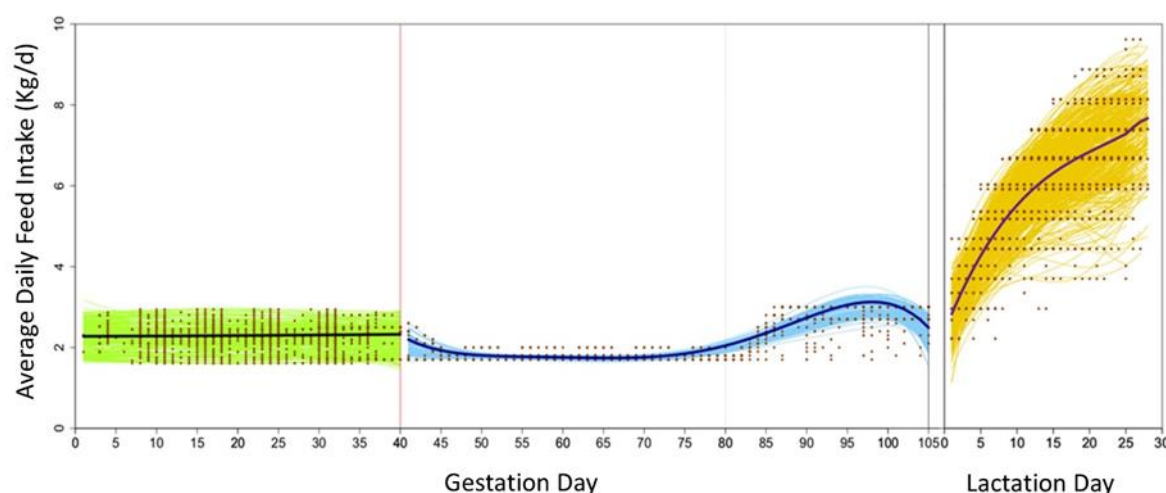

Figure 3. Average daily feed intake recorded in the Duroc dataset, and modelled via Legendre polynomial functions to retrieve missing values.

These traits were used to compute lactation efficiency in this population. Traits involved in lactation efficiency quantify energy inputs and outputs during lactation. Energy sources for a lactating sow are feed intake (daily lactation feed intake; FI_{lac}) and changes of body reserves during lactation (i.e., resulting in bodyweight and backfat changes). Available energy can be used for sow growth and maintenance, and for milk production. Milk production is usually quantified by litter weight gain. Therefore, daily changes in sow weight and daily backfat (i.e., total change divided by the duration of the lactation) are variables that quantify the balance of body reserves during lactation, which is negative whenever the sow loses weight and/or fat, and positive otherwise. Other traits involved in the definition of lactation feed efficiency are pre-farrow traits, which are those measured before farrowing (i.e., sow weight, backfat thickness and litter weight at farrowing) that may have an impact on sow lactation performance and are included as covariates in the analysis of all other traits. All these traits were combined as proposed by Gilbert *et al*. (2012) to produce a sow residual feed intake (sow RFI) indicative of the sow efficiency during lactation.

Results. Heritability estimates for feed intake traits were generally low, and ranging from 0.025 to 0.069 for ADFI during gestation. For lactation ADFI, heritability was higher and estimated at 0.12, which is lower than previously published heritabilities for this trait (0.26 Gilbert *et al*., 2012, to 0.30 Bergsma *et al*., 2008).
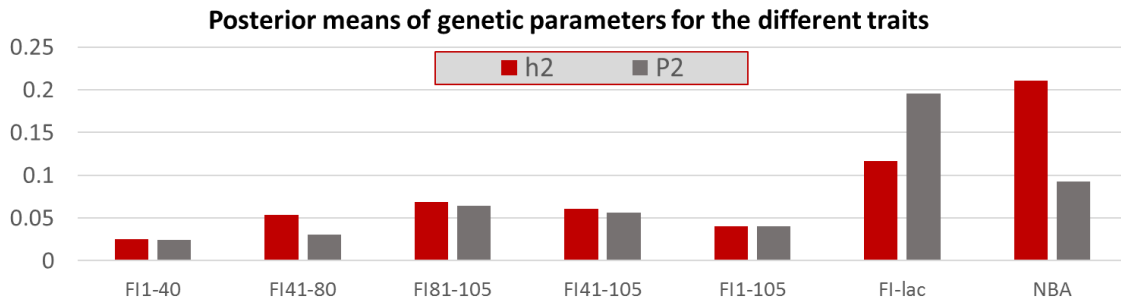
Posterior means of genetic parameters for the different traits

Figure 4. Posterior means of the variance component parameters (h2= heritability, P2=proportion of variance explained by the permanent environment of the sow) estimated for the feed intake traits during gestation and lactation.

Positive genetic correlations were obtained between feed intake during mid-late gestation and FI$_{lac}$ (Table 8). Positive genetic correlations were obtained between feed intake during early-middle gestation (0.99-0.64) traits and the number of piglets born alive. Nearly null genetic correlation between the number of piglets born alive and FI$_{lac}$ was obtained (0.09).

Table 8. Posterior means of genetic, permanent and residual correlations of feed intake and the number of born alive during lactation with feed intake during the previous gestation (posterior standard deviations are given in parenthesis).

	Genetic		Permanent		Residual	
	FI$_{lac}$	**NBA**	**FI$_{lac}$**	**NBA**	**FI$_{lac}$**	**NBA**
FI$_{1-40}$	0.14 (0.27)	0.99 (0.0.1)*	-0.78 (0.27)*	0.37 (0.49)	-0.03 (0.05)	-0.02 (0.04)
FI$_{41-80}$	0.63 (0.31)*	0.64 (0.31)*	-0.26 (0.59)	0.11 (0.61)	-0.02 (0.06)	-0.04 (0.05)
FI$_{81-105}$	0.82 (0.25)*	0.45 (0.30)	-0.31 (0.51)	0.39 (0.58)	-0.01 (0.06)	-0.06 (0.05)
FI$_{41-105}$	0.81 (0.27)*	0.45 (0.31)	-0.10 (0.42)	0.50 (0.63)	-0.02 (0.06)	-0.06 (0.05)
FI$_{1-105}$	-0.35 (0.54)	0.68 (0.26)*	-0.74 (0.31)*	0.27 (0.53)	0.04 (0.05)	0.01 (0.04)

FI$_{lac}$: feed intake during lactation; NBA: number of piglets born alive; FI$_{n-m}$: feed intake between days n and m of gestation.
* Values significantly different from zero.

Genetic parameters for lactation efficiency and related traits showed low to moderate heritability for feed efficiency during lactation (sow RFI heritability posterior mean [posterior sd] = 0.14±0.06) and its components (Table 9). The highest values were found for daily changes in body weight of the sow (0.28±0.08) and the litter (0.22±0.05). Both FI$_{lac}$ and change of backfat had a low heritability (<0.14). The lower value found for FI$_{lac}$ in this study compared with previously reported values is probably due to the inaccuracy of our measurements, which was conditioned by the way feed was supplied. In previous studies, data were recorded daily, whereas they were recorded for one or two days per week and predicted with a nonlinear model for the other days in our study. Given the noise in the daily-recorded data, the resulting prediction might not be accurate enough to compensate for the missing structure. Another important difference is that our heritability estimate of sow RFI is slightly higher than that of FI$_{lac}$, while in the aforementioned study (Gilbert *et al.*, 2012) the former reverse was observed. Sow RFI results from the difference between FI$_{lac}$ and predicted lactation feed intake based on traits defining energy and nutrient requirements of the lactating sow. The covariance structure between FI$_{lac}$ and the predictor traits for the sow requirements were different in our study compared to those reported in other lines and studies (Bergsma *et al.*, 2008; Gilbert *et al.*, 2012). The low heritability estimate for the change of backfat during lactation could be explained by a low accuracy in the measurement of the backfat thickness. In addition to the classical operator effect on this trait, it is particularly difficult to record in furry animals, such as is the Duroc population. The resulting

measurement error for backfat thickness would be 1 to 1.5 mm, which is 40 to 60% of backfat thickness balance variability during lactation. The proportions of phenotypic variance due to the permanent effect of the sow were moderate for energy inputs (i.e. lactation feed intake, changes in sow bodyweight and backfat) and low for daily litter weight gain and sow residual feed intake.

Table 9. Posterior means of variance components and ratios of phenotypic variance (posterior standard deviations are given in parenthesis).

Parameter[2]	FI_{lac}[1]	dSWB[1]	dBFB[1]	dLWG[1]	Sow RFI[1]
σ_a^2	0.014 (0.005)	0.059 (0.018)	0.0001 (0.0003)	0.015 (0.004)	0.016 (0.007)
σ_p^2	0.027 (0.006)	0.049 (0.016)	0.0001 (0.0003)	0.009 (0.003)	0.012 (0.007)
σ_e^2	0.119 (0.009)	0.102 (0.010)	0.002 (0.0001)	0.045 (0.003)	0.087 (0.007)
h^2	0.088 (0.029)	0.279 (0.076)	0.133 (0.042)	0.216 (0.052)	0.141 (0.061)
p^2	0.169 (0.035)	0.234 (0.077)	0.155 (0.031)	0.131 (0.049)	0.104 (0.064)

[1] FI_{lac}: daily lactation feed intake; dSWB: daily sow body weight balance; dBFB: daily backfat thickness balance; dLWG: daily litter weight gain; RFI = residual feed intake.
[2] σ_a^2: Additive variance; σ_p^2: Permanent variance; σ_e^2: Residual variance; h^2= heritability; p^2: permanent environmental variation relative to phenotypic variation.

Phenotypically, FI_{lac} was positively associated with daily balances of energy and nutrient reserves and litter weight gain (Table 10). Therefore, the more a sow eats during lactation the less she mobilises body reserves (i.e., resulting in body weight and backfat thickness changes) and the more she provides nutrients for litter weight gain. An increase in sow body weight changes was to an associated increase in changes in backfat (0.32±0.04), and to a decrease in litter weight gain (-0.26±0.04). In the same way, an increase in backfat thickness corresponded to a decrease in litter weight (-0.17±0.04). This means that increased mobilisation of body reserves is related to increased litter growth. Phenotypic and environmental correlations between FI_{lac} and change of backfat thickness were null.

Table 10. Posterior means (posterior sd) of phenotypic (r_p, above the diagonal) and genetic (r_g, below the diagonal) correlations.

$r_g\backslash r_p$	FI_{lac}	dSWB	dBFB	dLWG
FI_{lac}[1]	1	0.289 (0.034)	0.056 (0.038)	0.171 (0.035)
dSWB[1]	0.306 (0.197)	1	0.324 (0.037)	- 0.258 (0.037)
dBFB[1]	- 0.745 (0.124)	0.192 (0.206)	1	- 0.171 (0.037)
dLWG[1]	0.255 (0.199)	- 0.441 (0.192)	- 0.082 (0.197)	1

[1] FI_{lac}: daily lactation feed intake; dSWB: daily sow weight change; dBFB: daily backfat thickness change; dLWG: daily litter weight gain.

Daily lactation feed intake was highly correlated with change of backfat thickness (-0.75±0.12, Table 10). The genetic correlation between sow body weight change and litter weight gain was negative and moderate (-0.44±0.19). Daily sow weight and backfat thickness changes were phenotypically but not genetically correlated. The precision of our estimates of genetic correlations was low because of the limited number of records. Other genetic correlations were low and not statistically different from zero.

Conclusion. This study is a first attempt to obtain feed intake traits during gestation and lactation in a Duroc population, to estimate genetic parameters of traits involved in feed efficiency during lactation. The Duroc population of pigs has a genetic origin very different from those previously studied, which were Large White based populations (Gilbert *et al*., 2012; Bergsma *et al*., 2008). The restricted feeding applied during gestation resulted in a low variability of gestation feeding traits, with little room to study the genetic variance during gestation. During lactation, trait heritabilities were low to moderate, and

Feed-a-Gene

their magnitude could be high enough to guarantee a positive response to selection to improve feed efficiency during lactation. However, the limited accuracy of our estimates suggests that complete daily records of feed intake would help to reach higher accuracies

- o Recording feed intake during lactation in Iberian sows – variability and relation with litter traits in alternative lines

Iberian pigs are a local population of pigs used for their excellent meat quality. Until recently, they have not been subjected to any efficiency index assessment. The modern Iberian production system relies on piglet production farms that have the same characteristics as conventional piglet production facilities. From these maternity conventional farms, weaned piglets are moved to growing-fattening facilities, which pertain to one of the two following systems: combining two intensive growing and fattening phases, based exclusively on conventional feeds, or a growing phase with conventional feeds and a fattening period in the "Dehesa", where pigs have access to grass and acorns. In the first system, crossbred Iberian x Duroc animals are used, while in the other system, purebred Iberian are normally used. We aimed to characterize the lactation efficiency of Iberian sows and to explore the potential value of different lactation traits in breeding goals of the population.

Experimental design. Sows from two different Iberian lines were tested: 219 "Entrepeladas" (EE) and 229 "Retintas" (RR). Both groups were housed in the same farm, in the conventional system common to the maternity farms. A total of 1,157 lactation records were obtained, distributed in 30 batches. In each lactation, body weight and backfat thickness of the sow at the entrance to the maternity and at weaning, litter weight at parturition and at weaning, and prolificacy measurements (i.e., total born, number born alive and number of live piglets at weaning) were recorded, plus sow and piglet feed intake during the lactation. The heritability of all traits were estimated separately in the two populations; then they were combined into different lactation efficiency traits. Repeatability linear animal models adjusted for the body weight and backfat thickness of the sow at farrowing and for a year-season, and sow parity effect were used to estimate the genetic parameters.

Table 11. Descriptive statistics and variance components estimates for Entrepelada line (EE).

Trait (unit)	Mean	s.d.	n	Variance genetic	permanent	residual	h²
Total born (piglets)	8.61	2.07	524	1.32E-01	5.65E-01	3.35	0.03
Born alive (piglets)	8.26	1.99	524	5.68E-02	4.40E-01	3.22	0.02
Number at weaning (piglets)	7.13	1.08	505	5.94E-02	8.74E-02	1.01	0.05
Lactation length (d)	25.94	4.34	505	--	--	--	--
Backfat thickness at farrowing (mm)	31.75	12.57	487	--	--	--	--
Backfat thickness at weaning (mm)	28.44	12.23	479	6.45	2.37	23.6	0.20
Backfat thickness change (mm/d)	-0.14	0.24	420	4.52E-03	2.63E-04	3.92E-02	0.10
Sow body weight at farrowing (kg)	141.86	21.81	524	--	--	--	--
Sow body weight at weaning (kg)	136.15	19.73	515	7.19	3.37	43.6	0.13
Daily body weight change (kg/d)	-0.22	0.34	499	1.05E-02	5.23E-03	6.53E-02	0.13
Litter weight at farrowing (kg)	10.12	2.35	384	9.02E-01	2.18E-01	3.81	0.18
Litter weight at weaning (kg)	37.57	9.49	505	9.20	3.97	68.4	0.11
Piglet body weight gain (kg/d)	0.16	0.03	376	1.70E-04	7.66E-06	8.16E-04	0.17
Sow lactation feed intake (kg)	106.10	20.08	509	7.90E-01	1.47E+01	348	0.00
Sow lactation feed intake (kg/d)	4.10	0.21	501	1.32E-03	1.56E-03	4.02E-02	0.03
Lactation feed conversion ratio	4.20	1.82	373	1.46E-02	9.89E-02	3.23	0.00

Results and Discussion. Tables 11 and 12 show the descriptive statistics of the analysed traits in each line, and the variance components for the considered traits. The Entrepelada line had lower prolificacy (total born and born alive) than the Retinta line. However, the superior maternal abilities of the Entrepelada line led to slightly greater number of weaned piglets and greater litter weight gain. This was consistent with earlier studies in these lines (Noguera *et al*., 2016, Ibañez-Escriche *et al*., 2014). The daily feed intake in Entrepelada sows was 120 grams per day more than in the Retinta sows (i.e., 0.5 phenotypic standard deviation. The lactation FCR was 10% better in Entrepelada sows than in Retinta sows (4.20 vs 4.69 kg feed/kg litter gain). The Retinta sows were slightly lighter than the Entrepelada sows, but given the large variability of individual body weights, the average difference (3-4 kg) was not significant. In both lines, the body weight change during lactation was similar. The Retinta sows were slightly fatter than Entrepelada females, and lost less backfat during lactation (2.1 and 3.3 mm for Retinta and Entrepelada sows, respectively).

Table 12. Descriptive statistics and variance components estimates for Retinta line (RR).

Trait (unit)	Mean	s.d.	n	Variance			h²
				genetic	permanent	residual	
Total born (piglets)	9.04	2.14	541	9.50E-02	5.49E-01	3.67	0.02
Born alive (piglets)	8.65	2.01	541	8.87E-02	4.31E-01	3.37	0.02
Number at weaning (piglets)	6.90	1.10	525	1.05E-01	6.03E-03	1.12	0.09
Lactation length (d)	26.61	4.65	525	--	--	--	--
Backfat thickness at farrowing (mm)	32.22	12.24	512	--	--	--	--
Backfat thickness at weaning (mm)	30.13	12.23	491	4.28E+00	5.84	17.1	0.16
Backfat thickness change (mm/d)	-0.10	0.20	445	4.23E-03	4.70E-04	2.44E-02	0.15
Sow body weight at farrowing (kg)	138.62	17.55	540	--	--	--	--
Sow body weight at weaning (kg)	132.43	16.63	531	5.89E-01	12.7	40.2	0.01
Daily body weight change (kg/d)	-0.24	0.33	519	8.48E-04	1.71E-02	6.73E-02	0.01
Litter weight at farrowing (kg)	10.34	2.30	379	7.65E-02	8.61E-01	3.85	0.02
Litter weight at weaning (kg)	35.88	9.46	525	3.15E-01	6.02	80.9	0.00
Piglet body weight gain (kg/d)	0.15	0.04	370	1.08E-04	2.19E-05	1.14E-03	0.09
Sow lactation feed intake (kg)	108.95	20.85	525	1.33E+01	9.37E-01	399	0.03
Sow lactation feed intake (kg/d)	4.08	0.26	518	1.23E-03	4.94E-05	6.10E-02	0.02
Lactation feed conversion ratio	4.69	1.96	365	9.59E-03	2.55E-01	3.60	0.00

Only backfat related traits had heritabilities different from zero in both lines. In the Entrepelada line, the sow body weight at weaning and body weight change also had significant heritabilities. In this line, litter weights and piglets growth rates had heritabilities estimates greater than 0.1. Feed intake traits did not show relevant heritability (<0.03). Thus, based on our results, no direct improvement of lactation efficiency could be obtained in these lines. Nevertheless, improvement of backfat traits, which are heritable in both lines, may indirectly benefit feed efficiency traits. Given the number of records, the accuracy of correlation estimates would be too low to conclude on this aspect and additional data will be needed to propose a selection strategy on these aspects.

Conclusion. The two Iberian populations studied differed in lactation performance: one had better prolificacy, whereas the other one had better maternal behaviour. The tested lactation feed efficiency trait was not heritable and cannot be proposed as a selection criterion in these lines. However, other traits contributing to lactation efficiency showed significant heritabilities, so alternative strategies via indirect improvement of efficiency can be envisaged.

3.1.2   Behaviour and activity

Activity of growing animals can be seen as an element of welfare when positive behaviour is expressed (e.g., when the animal explores its environment or interacts with pen mates), but also as a source of energy expenditure at the expense of feed efficiency (Meunier-Salaun *et al.*, 2014). Understanding how these traits (i.e., welfare, activity, and efficiency) interact at the genetic level is thus a key to contribute to improving feed efficiency while not impairing them. However, measuring activity remains a challenge. In task 2.2 of the project, effort have been dedicated to measuring activity in group-housed animals through video recording. However, no satisfactory way could be found to ensure individual identification, a key element in genetic studies. The genetics of behaviour and activity in relation to feed efficiency were thus explored via two indirect measurements: recording of body lesions (as a proxy for aggressive behaviour) and recording feeding behaviour using electronic feeders, with the

objective to evaluate their potential to contribute to a more accurate selection for feed efficiency. When possible, indicators of the social hierarchy in the pen were evaluated as potentially novel traits contributing to feed efficiency. A Duroc population was used to assess the two types of records, based on developments made in WP2 of the project, whereas a conventional population was used to assess feeding behaviour only.

- ➢ Aggressiveness and feed efficiency in pigs

Experimental design. Body lesions were recorded 3-4 times during the fattening period, following the welfare quality protocol (http://www.welfarequalitynetwork.net/media/1018/pig_protocol.pdf). Each individual pig was scored for the presence of lesions on the ears, head, body, hind-quarter, and legs. In two batches, all the animals in a number of pens were recorded, while in two other batches, 3 to 4 animals were scored in each pen. For each animal, the number of lesions at each location and the total number of lesions were analysed, resulting in a total of 304 lesion scores (Table 13).

Table 13. Average number of lesions recorded per pig in each batch at each location.

Batch	N	Ear	Head	Body	Hind-quarters	Legs
1	67	0.79	0.19	0.33	0.15	0.16
2	47	0.83	0.64	0.40	0.23	0.02
4	63	0.59	0.35	0.35	0.25	0.02
5	127	0.26	0.17	0.24	0.10	0.01

Table 14. Descriptive data on interactions recorded between pigs.

Batch	N	Fight		Head-hitting		Bite		Chase	
		Initiated	Received	Initiated	Received	Initiated	Received	Initiated	Received
1	67	0.16	0.16	2.27	2.27	0.58	0.55	0.06	0.06
2	68	0.00	0.00	0.49	0.50	0.09	0.09	0.04	0.04
4	70	0.13	0.14	1.39	1.36	0.40	0.41	0.00	0.00
5	127	0.10	0.10	1.08	1.10	0.29	0.28	0.02	0.02

During the fattening period, three to four interaction records were obtained per pen. Each pen was evaluated for 20 minutes, during which all antagonistic interactions are registered. The following interactions were considered for each animal initiating or receiving an action: chasing, biting, fighting, and head hitting. For all animals involved in the different interactions, the total number of initiated or received actions were obtained. Records for a total of 332 individuals were available (Table 14).

Table 15. Descriptive statistics of lesions and interaction traits (initiated or received), and genetic parameters (heritability, genetic, and phenotypic correlations with residual feed intake).

Trait	Mean*	s.d.	n	h²	Genetic correlation	Phenotypic correlation
Sum of the lesions	2.33	1.66	304	0.02	-0.74	0.02
Lesions in the ear	1.53	0.91	304	0.01	0.64	-0.02
Lesions in the head	1.28	0.69	304	0.04	-0.88	-0.04
Lesions in the body	1.31	0.74	304	0.03	-0.69	0.02
Lesions in the hind-quarters	1.16	0.53	304	0.02	-0.77	0.10
Lesions in the legs	1.05	0.24	304	0.64	-0.09	-0.02
Initiated fight	1.10	0.39	332	0.10	0.40	0.01
Received fight	1.10	0.37	332	0.02	0.22	-0.07
Initiated head hit	2.26	1.73	332	0.06	0.39	0.12
Received head hit	2.27	1.75	332	0.10	0.72	0.10
Initiated bite	1.33	0.74	332	0.02	0.54	0.03
Received bite	1.33	0.70	332	0.01	0.63	0.03
Initiated chase	1.03	0.19	332	0.09	-0.32	0.06
Received chase	1.03	0.17	332	0.31	0.19	0.07
log(Sum of the lesions)	4.91	4.42	304	0.01	-0.49	0.04
log(Lesions in the ear)	6.98	4.29	304	0.01	0.39	-0.02
log(Lesions in the head)	8.35	3.51	304	0.06	-0.88	-0.04
log(Lesions in the body)	8.17	3.66	304	0.03	-0.59	0.04
log(Lesions in the hind-quarters)	8.95	2.93	304	0.02	-0.66	0.08
log(Lesions in the legs)	9.63	1.80	304	0.59	-0.13	-0.02
log(Initiated fight)	9.31	2.43	332	0.07	0.54	0.02
log(Received fight)	9.22	2.57	332	0.02	0.10	-0.07
log(Initiated head hit)	5.26	4.45	332	0.03	-0.19	0.10
log(Received head hit)	5.12	4.45	332	0.09	0.63	0.08
log(Initiated bite)	8.05	3.74	332	0.03	0.71	0.04
log(Received bite)	7.87	3.88	332	0.02	0.77	0.03
log(Initiated chase)	9.75	1.51	332	0.08	-0.34	0.06
log(Received chase)	9.72	1.59	332	0.30	0.19	0.07

*All trait values were added 1.0001 (e.g., no lesions in the ear was coded as 1.0001, 1 lesion in the ear was coded as 2.0001), to account for zero values in the descriptive statistics of the records and log transformed values.

Bivariate linear mixed models combining these behavioural traits with a feed efficiency trait (i.e., RFI) were fitted to estimate their genetic relationships with feed efficiency. All models included the fixed effects of the batch and sex (i.e., female or castrated male), the covariates of age at live weight recording and number of pen mates, and the random litter, pen, and additive genetic effects. Heritabilities of behavioural traits and their genetic correlation with residual feed intake were estimated. The traits were considered in their original scale (counts) and also transformed to a logarithmic scale to better fit the assumption of Gaussian residuals.

Results and Discussion. The most frequent lesions were observed on the ears (Table 13), with an average of 0.53 lesions per pig. Considering ear lesions as a binary trait (because each animal has or does not have a lesion), ear lesions were observed in 33% of the pigs. For head and body lesions, the frequency was around 20%, and hind-quarter lesions occurred with a frequency of 11%.

The action that more frequently occurred were the hits with the head (Table 14). One particular animal started this antagonist behaviour up to 10 times during the observation period. If the trait is treated

as a binary trait (an animal providing or not at least one hit with its head to another pig), its frequency was as high as 53%. The second most frequent antagonistic action was biting, with a frequency of 21%.

Table 15 presents raw averages and standard deviations of the lesions and behavioural traits. The heritability estimates and genetic correlations with RFI are also shown. In the different bivariate analyses, all the available RFI data were considered. Similar genetic parameters were obtained for the traits with their original units (counts) and the log-transformed traits. The highest estimated heritability (0.64) was estimated for the lesions in the legs, which had very low incidence, so it should be considered with caution, as estimates for initiated and received chasing events. The phenotypic correlations of other lesions traits with residual feed intake were close to zero, and their heritabilities are lower than 0.05. Because the genetic variances were small for these traits, the genetic correlations with residual feed intake were estimated with very low accuracy and should not be further considered.

Similar conclusions arose from estimates of interaction traits, but for the number of received head hits, which had the highest incidence (Table 14). For this trait, the heritability was 0.10, and the genetic and phenotypic correlation estimates with residual feed intake were positive. Thus, the less genetically efficient animals would carry over genetic effects that increase the incidence of receiving head hits from their pen mates. No clear antagonist genetic correlation between RFI and initiated head hits was identified, with a genetic correlation positive on the natural trait scale and a negative and low correlation (-0.19) on the logarithm scale. No reciprocal relationship with feed efficiency was apparent for that type of behaviour.

Conclusions. These preliminary analyses show that visually recorded lesions and antagonistic behaviours are of limited value to improve feed efficiency through genetic selection. Only the most frequent antagonistic behaviour (i.e., number of received head hits) provided a relevant genetic correlation with feed efficiency. Nevertheless, statistical analyses accounting for the trait distribution specificities (e.g., large number of zeros) could reveal additional useful genetic variability.

➢ Feeding behaviour and social hierarchy in group housed animals

Feeding behaviour traits are automatically recorded by electronic feeders on animals raised in groups. They reflect a within-pen dynamics of eating behaviour. From a genetic perspective, it could be possible to use this information either through a relevant genetic correlation with other performance traits, or because the phenotypic/genetic relationship between these traits explains the within-pen social structure of the animals.

o Feeding behaviour and feed efficiency in Duroc pigs

Experimental design. The study was conducted in a Duroc population (N=1,144 pigs from 10 batches), using traits obtained using electronic feeders. The base records were the visit duration to the feeder and the associated feed intake, together with the time when the visit started for each pig visiting the feeder. The traits computed for the feeding behaviour analysis were average daily eating rate, daily feeding frequency, occupation time, and time between consecutive visits. Table 16 presents descriptive statistics of these traits, together with statistics for four base performance traits.

Table 16. Descriptive statistics for performance and feeding behaviour traits.

Trait	Min	Mean	Max	s.d.
Behaviour				
Eating rate, g/min	15.28	38.60	65.14	7.41
Occupation time, min/d	33.02	60.72	103.49	10.27
Feeding frequency, visits/d	3.54	10.11	24.88	2.98
Time between visits, h	1.64	3.93	9.89	1.03
Performance	0.22	0.82	1.07	0.09
Daily gain, kg/d				
Feed intake, kg/d	0.86	2.31	3.67	0.37
FCR	2.07	2.77	3.89	0.24
Backfat thickness, mm	6.44	18.19	32.74	4.40

Result and Discussion. Heritabilities of feeding behaviour traits were moderate to high. They were all higher were than 0.23 (Table 17) and in agreement with previous estimates of this type of traits in pigs (Young *et al*., 2011; Do *et al*., 2013; Lu *et al*., 2017).

The social structure was not directly recorded. However, based on the phenotypic correlations among feeding behaviour traits (Table 17) animals can be clustered into two groups: 1) Animals that have a low eating rate, occupy the feeder for a long time, have a large number of visits per day, and with short intervals between visits, 2) Animals that eat fast, spend less time at the feeder, have a reduced number of visits per day, and long intervals between visits.

Only two genetic correlations among feeding behaviour traits were different from zero (Table 15). It concerned the correlation between eating rate and occupation time (-0.76±0.16) and the correlation between feeding frequency and time between visits (-0.78±0.09). The correlations of feeding intervals with feeding rate and occupation time were negative, so the previously described patterns at the phenotypic level might not completely hold at the genetic level. We could postulate that some animals might carry genetic effects to stay longer in the feeder and eat at a low rate, while others carry genetic effects for having reduced feeding intervals, a large number of visits, and potentially a low eating rate and occupation time. Combined with behaviour data, it could be possible to decipher a social hierarchy in some of these pens, and thus evaluate if the described feeding patterns can be related to dominant or passive behaviours.

Table 17. Heritability (diagonal) estimates and genetic (above the diagonal) and phenotypic (below the diagonal) correlation estimates, with their standard errors in parenthesis.

Trait	ER	OT	FF	Fint	ADG	ADFI	FCR	BF
ER	0.30 (0.08)	-0.76 (0.16)*	0.22 (0.20)	-0.31 (0.20)*	0.60 (0.24)*	0.64 (0.22)*	0.40 (0.29)	0.53 (0.24)*
OT	-0.65 (0.02)*	0.23 (0.10)	0.27 (0.25)	-0.33 (0.26)*	-0.24 (0.38)	-0.30 (0.37)	-0.28 (0.39)	-0.33 (0.36)
FF	-0.11 (0.04)*	0.20 (0.04)*	0.48 (0.09)	-0.78 (0.09)*	0.11 (0.26)	0.16 (0.24)	0.15 (0.25)	0.17 (0.22)
FInt	0.10 (0.04)*	-0.28 (0.03)*	-0.60 (0.03)*	0.47 (0.08)	-0.11 (0.28)	-0.09 (0.25)	0.04 (0.28)	-0.32 (0.23)
ADG	0.38 (0.03)*	0.16 (0.04)*	-0.11 (0.04)*	0.10 (0.04)*	0.19 (0.08)	0.80 (0.13)*	0.14 (0.41)	0.51 (0.25)*
ADFI	0.42 (0.03)*	0.20 (0.04)*	-0.11 (0.04)*	0.04 (0.04)	0.82 (0.01)*	0.22 (0.08)	0.66 (0.22)*	0.64 (0.16)*
FCR	0.16 (0.04)*	0.11 (0.04)*	-0.03 (0.04)	-0.05 (0.04)	-0.05 (0.04)	0.52 (0.03)*	0.21 (0.09)	0.41 (0.33)*
BF	0.29 (0.03)*	0.16 (0.04)*	-0.09 (0.04)*	-0.04 (0.04)	0.59 (0.03)*	0.68 (0.02)*	0.31 (0.03)*	0.32 (0.10)

ET: eating rate; OT: occupation time; FF: feeding frequency, Fint: interval between successive visit; ADG: average daily gain; ADFI: average daily feed intake; FCR: feed conversion rate; BF: backfat thickness.
* indicate values differing from zero.

Among the feeding behaviour traits, only eating rate was significantly correlated with production traits, except with FCR. A higher eating rate would be related at the genetic level with increased ADG, ADFI, and backfat thickness. It is thus the only feeding behaviour trait that could add relevant information to improve the accuracy of the genetic evaluation for performance traits other than FCR. Given the magnitude of the standard error, recording additional data might help consolidating a slightly positive genetic correlation with FCR that could be used. Eating rate is related with the feeding activity level of the pigs, but also with the passage rate in the digestive tract. This trait may be considered for further improvement of feed efficiency.

Using the same dataset, Ragab *et al.* (2018) reported that including feeding behaviour traits in models to fit indirect genetic effects can slightly improve the predictive ability for production traits. The feeding behaviour traits were used to establish the degree of interaction among animals in a pen, the differential degree of interaction being proportional to the Euclidean distance between the animals based on their feeding behaviour traits. The details of these results are reported in deliverable D5.3 of the project.

Conclusions. The phenotypic relationships between feeding behaviour traits distinguishes two groups of feeding behaviours that could reflect a social hierarchical structure. Only eating rate was correlated with performance traits in this Duroc population and this trait could later be used in a multivariate genetic evaluation to improve the accuracy of the evaluations of performance traits. The alternative is to use feeding behaviour traits to improve the prediction accuracies by fitting indirect genetic effects, providing indicators of the animal's degree of interaction with their pen mates.

o   Effects of feed intake behaviour on feed efficiency in pigs

The aim of this study was to develop an additional feed intake behaviour trait, which could be used in breeding programs to improve feed efficiency. First, the genetic parameters for feeding behaviour traits recorded during the fattening period were estimated from electronic feeder records. Second, a rank index was developed based on these records: it assumed that when the interval between two feed intake events was smaller than four seconds, the first individual was chased away by the individual that then started eating. This interpretation is possible only with electronic feeders that do not close behind the eating pig, so that animals can be disturbed while eating.

Data recording. Data of feeding station from five Topigs Norsvin pig-farms were used. The number of pens equipped with electronic feeding stations per farm varied from 40 to 130. The records included 4,214 groups of pigs. In this study, a group indicates a pen of animals that have the same start-date of the fattening period. Data were recorded on a Large White sire line and their offspring from different type of commercial crossbred sows. Three farms had registrations for the sire line only; the other two had registrations for both purebred and different crossbreds.

The data contained information of more than 37,710 individuals. Nearly 80% of the individuals were purebred animals. The data included information about the identification of the pig, farm number, room and pen within the farm, gender, birthdate, parity number, breeding line, start test date, end test date, start- and end weight of the pig for the test-period, number of test days, daily feed intake, number of meals and visits, time spent eating and visiting the electronic feeding stations, test growth rate, back fat thickness, and feed conversion ratio (Table 18).

Table 18. Descriptive statistics for production and feeding behaviour traits during the fattening period.

	Mean	s.d.	Minimum	Maximum
Start weight (kg)	31.9	8.6	14.0	87.0
End weight (kg)	121.9	11.9	50.0	173.0
N of test days used in analysis	61	24	1	180
Daily feed intake (g)	2448	521	200	5230
Average daily gain (g per day)	1029	141	338	1571
Back fat thickness (mm)	9.90	2.27	4.00	25.3
Feed conversion ratio	2.44	0.40	1.01	5.00
Eating rate (g/min per day)	63.6	25.6	2.9	299.8
Number of visits (per day)	22.2	14.2	1.0	214.4
Visit time (min per day)	43.72	16.84	2.57	214.39
Number of meals (per day)	11.5	6.6	1.0	90.2
Meals time (min per day)	43.35	16.50	3.11	209.68

Results. Heritabilities ranged from 0.10 (visiting time and meals time) to 0.38 (backfat thickness). The genetic correlations ranged from -0.63 between feeding rate and meals time (very close to -0.76 estimated in Duroc for similar traits) to 0.78 between daily feed intake and average daily gain (very close to 0.80 estimated in Duroc for a similar trait). Subsequently, the fattening period was divided into two periods (i.e., from day 1 until day 53, and from day 54 until the end of the fattening period), to estimate the genetic parameters in the different periods and potentially capture changes in the feed behaviour dynamics with age. Heritabilities for period 1 ranged from 0.12 to 0.26, and for period 2

Feed-a-Gene

from 0.17 to 0.28, suggesting that values for traits in the second period are slightly more genetically determined. Estimates of the genetic correlations for the same traits in the two periods were computed and they ranged from 0.86 to 0.94. Therefore, the traits were considered as very similar in both periods and were not distinguished afterwards.

The rank index for every individual was calculated by the formula of Puppe *et al*. (2008), ranking each animal within a pen. The pen size varied from 6 to 16 individuals, so the Blom's method was used to correct the rank for the group size. Based on this rank value, individuals were divided into three groups. Group 1 consisted of the 20% most dominant animals (i.e., animals that chase other individuals away from the feeder), group 3 consists of the 20% most docile individuals (i.e., animals chased away by others), and group 2 consists of the 60% middle-ranked individuals. From the phenotypic analyses, it appeared that individuals of the first group had the lowest feed intake per meal, lowest time per meal, and lowest average daily gain. The group with the most docile individuals had the best FCR and highest eating rate. Subsequently, the standard deviation of the rank index within each pen was computed. The pens with the highest standard deviation had the highest feed intake per meal, the lowest time per meal, and the highest eating rate and FCR on average. Finally, the genetic parameters for the rank index were calculated. The heritability for the Blom's rank score was 0.12. The genetic correlations confirmed the results of the phenotypic analyses (e.g., that a higher FCR corresponds to a high-ranked individual, interpreted as dominant).

In conclusion, data from electronic feeding stations can be used to compute a rank index that represents the hierarchy of the pigs in a pen. This information could be included in a breeding program to improve the feed efficiency.

3.1.3   Welfare and robustness indicators

Selection for more efficiency is often questioned as reducing the ability of farm animals to face stress and overcome challenges (Knap, 2009). To evaluate the genetic relationships between feed efficiency and welfare and robustness, two types of analyses were run. First, biological indicators of welfare developed in WP2 were submitted to genetic analyses in relation with feed efficiency so that they could be used to improve animal welfare through selection if adverse relationships are identified. Second, the relationships between robustness and feed efficiency were evaluated by comparing lines with contrasted abilities for these criteria.

➢   **Welfare indicators and feed efficiency in Duroc population**

Experimental design. Biological samples were collected at the beginning and at the end of the fattening period in the Duroc population tested at IRTA. Table 19 shows a description of the samples collection. Blood samples were used to obtain a complete hemogram, saliva samples were obtained to determine the concentration of chromogranin A, and hair samples were used to determine the cortisol concentration.

Table 19. Number of samples collected per batch depending on the sampling time.

Batch	Saliva		Hair		Blood	
	Start of fattening	End of fattening	Start of fattening	End of fattening	Start of fattening	End of fattening
1	66	66	0	0	65	67
2	0	0	0	0	0	0
3	20	20	0	0	0	0
4	62	64	63	64	62	62
5	0	64	-	-	62	55

Saliva: used for chromogranin A determination; Hair: used for cortisol determination; blood: used for hemogram analyses.

A total of 81 traits were derived from raw laboratory measurements. The individual counts and the differences between counts at start and end of the fattening period were tested for their genetic determinism and their genetic correlations with residual feed intake. When necessary, counts were log-transformed to respect residual normality. Descriptive statistics of the traits and heritability and genetic correlation estimates are given in tables 20 (chromogranin A and cortisol) and 21 (hemogram traits). The same bivariate mixed linear model as described in section 3.1.2 (subsection Aggressiveness and feed efficiency in pigs) was used.

Results and discussion. On average, cortisol measured in the hair, on the log scale, and chromogranin A showed a decreased average concentration between the beginning and the end of the fattening period.

Table 20. Descriptive statistics, heritabilities and genetic and phenotypic correlations with residual feed intake for chromogranin A (CGA) in saliva and cortisol in hair measured at the start and at the end of the fattening period.

Trait (units)	Mean	s.d.	n	h²	Genetic correlation	Phenotypic correlation
log(end cortisol), log(pg/mg)	4.49	1.47	64	0.12	0.38	0.10
log(start cortisol), log(pg/mg)	5.12	0.47	63	0.08	0.62	-0.08
Log(difference from end to start (cortisol)), log(pg/mg)	-0.62	1.59	63	0.02	0.14	0.11
End chromogranin A, µg/ml	0.50	0.60	214	0.02	0.70	0.07
Start chromogranin A, µg/ml	0.90	0.83	148	0.05	-0.20	0.05
Difference from end to start chromogranin A, µg/ml	-0.29	0.87	147	0.01	0.66	0.02
log(end chromogranin A), log(µg/ml)	-1.28	1.10	214	0.01	-0.25	0.02
log(start chromogranin A), log(µg/ml)	-0.55	1.02	148	0.08	-0.37	0.04
Log(difference from end to start chromogranin A), log(µg/ml)	-0.58	1.18	147	0.01	0.35	0.00

This study is the first assessment of the heritability of these two traits in pigs. Only the cortisol levels at the end of the fattening period had a heritability higher than 0.10. The estimated genetic correlations of cortisol with residual feed intake was positive at the beginning and the end of the test (i.e., animals with higher breeding values for cortisol would be less feed efficient).

Table 21. Descriptive statistics, heritabilities and genetic and phenotypic correlations with residual feed intake for hemogram traits measured at the start and at the end of the fattening period.

Trait (unit)	Mean	s.d.	n	h²	Genetic Correlation	Phenotypic Correlation
Start: Haematocrit, %	33.28	2.44	189	0.33	-0.06	0.09
Start: Haemoglobin, g/dL	10.54	0.86	189	0.29	-0.06	0.09
Start: Mean corpuscular volume, fL	51.05	3.20	189	0.60	-0.11	-0.08
Start: Mean corpuscular haemoglobin, pg	16.18	1.20	189	0.52	-0.11	-0.05
Start: Mean corpuscular haemoglobin concentration, g/dL	31.67	0.84	189	0.18	-0.15	0.05
Start: Erythrocytes,M cells/µL	6.54	0.52	189	0.70	0.06	0.15
Start: Leukocytes, K cells/µL	18.59	6.01	189	0.12	-0.83	-0.03
Start: Eosinophils, K cells/µL	0.31	0.20	170	0.18	-0.79	-0.11
Start: Lymphocytes, cells/µL	10.76	3.48	189	0.04	-0.82	-0.04
Start: Monocytes, K cells/µL	0.47	0.31	189	0.09	-0.18	0.04
Start: Neutrophils, K cells/µL	7.04	3.56	189	0.21	-0.54	0.00
Start Ratio: neutrophils to lymphocytes	0.70	0.38	189	0.38	0.04	0.00
End: Haematocrit, %	37.75	2.74	184	0.11	0.88	0.11
End: Haemoglobin, g/dL	12.46	0.94	184	0.11	0.88	0.12
End: Mean corpuscular volume, fL	51.98	4.22	184	0.27	-0.12	-0.03
End: Mean corpuscular haemoglobin, pg	17.16	1.42	184	0.08	-0.29	0.00
End: Mean corpuscular haemoglobin concentration, g/dL	33.02	1.01	184	0.11	0.02	0.04
End: Erythrocytes, M cells/µL	7.30	0.67	184	0.40	0.47	0.12
End: Leukocytes, K cells/µL	17.02	4.46	184	0.20	0.04	0.00
End: Eosinophils, K cells/µL	0.38	0.24	183	0.44	-0.51	-0.12
End: Lymphocytes, K cells/µL	10.73	3.48	184	0.29	0.45	0.05
End: Monocytes, K cells/µL	0.37	0.17	182	0.15	0.03	-0.06
End: Neutrophils, K cells/µL	5.53	1.86	180	0.22	-0.86	-0.09
End: Ratio neutrophils to lymphocytes	0.55	0.22	180	0.41	-0.82	-0.06
Diff. (End.Start) Haematocrit, %	4.45	3.11	179	0.09	0.78	0.02
Diff. (End.Start) Haemoglobin, g/dL	1.94	1.05	177	0.07	0.75	0.03
Diff. (End.Start) Mean corpuscular volume, fL	0.93	3.92	179	0.03	0.32	0.04
Diff. (End.Start) Mean corpuscular haemoglobin, pg	0.99	1.37	178	0.12	0.09	0.01
Diff. (End.Start) Mean corpuscular haemoglobin concentration, g/dL	1.36	1.31	179	0.13	0.06	-0.03
Diff. (End.Start)Erythrocytes, M cells/µL	0.76	0.68	178	0.06	0.74	0.01
Diff. (End.Start) Leukocytes, K cells/µL	-1.31	7.25	179	0.10	0.59	0.03
Diff. (End.Start) Eosinophils, K cells/µL	0.11	0.30	179	0.24	-0.31	-0.01
Diff. (End.Start) Lymphocytes, K cells/µL	0.02	4.65	179	0.10	0.87	0.07
Diff. (End.Start) Monocytes, K cells/µL	-0.09	0.35	179	0.07	0.26	-0.08
Diff. (End.Start) Neutrophils, K cells/µL	-1.32	3.93	176	0.20	-0.05	-0.02
Diff. (End.Start) Ratio neutrophils to lymphocytes	-0.14	0.42	173	0.30	-0.48	-0.01

However, standard errors were large and the values need confirmation. Previous work on monkeys (Fairbanks *et al.*, 2011) showed higher heritability estimates for cortisol in hair (around 0.3), which

could be related to different housing systems in the two studies: the conditions in our facilities are close to commercial farms, and probably far from the well-controlled low stress experimental environment of the monkey study. All heritabilities for chromogranin A traits were lower than 0.10, which points out a weak genetic control of this molecule in saliva compare do the environmental impact. As this measurement reflects acute stress episodes (Casal *et al.*, 2017), the impact of environmental factors could be much higher than the genetic control from the animal.

Most of hemogram traits had heritability estimates different from zero (Table 21). For most traits, genetic correlations with residual feed intake were different from zero and moderate to high. Heritability estimates associated to characteristics of red blood cells (i.e., haematocrit, haemoglobin traitsn and erythrocytes count) were substantially higher at the beginning of the fattening period than at the end, whereas white cells counts had higher heritabilities at the end of the fattening period, except the heritability of the ratio of neutrophils to lymphocytes, which remained constant. Nearly all white cells counts at the beginning of fattening period were strongly negatively correlated with RFI. At the end of the fattening period, some of these correlations were reduced or even changed sign, as for lymphocyte counts. At the end of the fattening period, the erythrocytes count, the haematocrit, and the haemoglobin count were positively correlated with RFI. At the end of the fattening period, the ratio of neutrophils to lymphocytes was negatively correlated with RFI, whereas the correlation at the beginning of the period was not different from zero. The heritabilities of the differences between records at the beginning and at the end of the fattening period were low for the red blood cell counts and moderate for white cells. Only the eosinophils and neutrophils counts and the ratio of neutrophils to lymphocytes had consistently moderate heritabilities at different moments. Estimations of heritabilities for hematological traits have been reported earlier with moderate estimates (Reiner *et al.*, 2007 and 2008; Ponsuksili *et al.*, 2016), and their association with feed efficiency traits have been previously shown in pigs divergently selected for feed efficiency (Jégou *et al.*, 2016). In this last study, lower red blood cell counts, haemoglobin, and haematocrit were associated to high RFI, which contrasts with the positive genetic correlations observed in our study.

Conclusions. Only some of the haematological traits showed clear genetic correlations with feed efficiency. The advantages of considering hair cortisol or saliva chromogranin A to improve the breeding value prediction accuracy for feed efficiency seemed quite low.

➢   Genetic of feed efficiency and robustness in pigs

Experimental design. The hypothalamic-pituitary-adrenocortical (HPA) axis, via the release of cortisol in blood by the adrenal gland, is a main actor in responses to stress (Mormede and Terenina, 2012). The measure of cortisol in blood after a standardised ACTH injection has been proposed as a measure of animal robustness. This study had two objectives: 1. To evaluate if selection for feed efficiency altered the activity of the HPA axis, by measuring plasma cortisol after an ACTH injection, and 2. to evaluate if a genetic difference in the HPA axis alters feed efficiency and production performance, and to evaluate how this performance would be affected when alternative dietary resources are available. Parallel trials were run under the same conditions on pigs genetically divergent for feed efficiency (i.e., ow RFI or high RFI) and for response to the ACTH injection (i.e., low cortisol or high cortisol). Four batches of 12 pigs per line were tested for their response to an ACTH injection at 6 weeks of age. Blood samples were collected before the ACTH injection, and 1 and 4 hours after the injection to measure cortisol and blood parameters (i.e., urea, glucose, free fatty acids, IGF-I), and to perform transcriptomic

studies to understand the underlying biology of the line differences and responses to the injection (see 3.2.2).

At 10 weeks of age, pens of 12 pigs were fed either a conventional diet (Control) with 9.7 MJ net energy and 160 g crude protein/kg or an alternative diet (Test) with a 10% reduction in net energy and the same ratios of amino acids to net energy, and of digestible amino acids to digestible lysine as the Control diet through the inclusion of feed resources with higher dietary fibre content. Animals had free access to feed and water during the test. Pigs were weighed at 10, 15, and 23 (BW23w) weeks of age. Individual ADG from 10 to 23 weeks, ADFI, FCR for this period were recorded. At 23 weeks of age, backfat thickness was measured by ultrasonic recording. At slaughter, the lean meat content of the carcass was computed from muscle and backfat thicknesses, and the carcass yield was calculated after cooling. A total of 187 pigs were allotted at 10 weeks of age, 181 had records for the growing period, and 171 had records at slaughter. Data from each pair of lines (i.e., RFI and cortisol) were analysed separately with linear models.
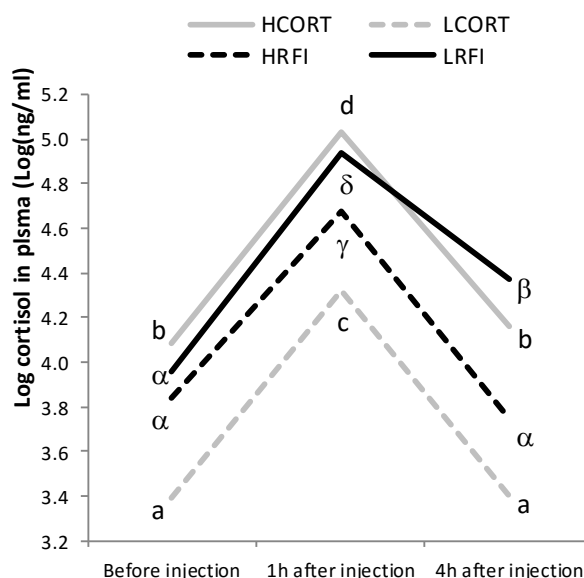
Results and discussion

Figure 5. Plasma cortisol measured before, one hour and four hours after a standardised ACTH injection in 6 weeks old piglets from lines selected for high (HCORT) or low (LCORT) plasma cortisol one hour after injection, and lines selected for low (LRFI) or high (HRFI) residual feed intake. Different letters indicate different values (P<0.05) between the cortisol lines (Latin letters) or between the RFI lines (Greek letters).

Response to ACTH. The two cortisol lines responded to the injection in a very classical manner, with an increase by a factor 2.3 of the plasma cortisol one hour after injection (on the measurement scale, i.e., +0.9 on the log scale), and a return to the basal level four hours after the injection (Figure 5). As a result of the divergent selection for three generations, the plasma cortisol differed at all times between these lines. The RFI lines had a similar basal level, but differed significantly at the two measurements after injection, with the low RFI line having higher cortisol levels than the high RFI line during the test. In addition, the low RFI pigs did not return to the basal level four hours after injection, which indicates an increased response of the adrenal gland to the ACTH injection in this line. Altogether, the initial

hypothesis of lower cortisol and lowered HPA activity in more efficient animals was not confirmed by this test.

Growth and feed intake. The RFI lines differed with the Control diet according to the selection objectives of the lines (i.e., low RFI pigs had lower ADFI and FCR than high RFI pigs; Table 22). No line effect was observed in the cortisol lines on growth and feed intake traits, which contrasts with the initial hypothesis of reduced production performance in high cortisol animals.

Table 22. Least square means[1] for line (High and Low) x diet (Control and Test) interaction on traits recorded during the growing period[2] on the cortisol (CORT) and RFI lines[3].

Trait	Line	Probability			High line		Low line	
		Line	Diet	Line x Diet	Control	Test	Control	Test
BW23w, kg	CORT	0.74	0.033	0.93	97.0[b]	94.3[a]	96.7[b]	93.7[a]
BW23w, kg	RFI	0.008	1x10^{-8}	0.06	101.2[c]	96.0[b]	100.2[c]	90.6[a]
ADG, g/d	CORT	0.77	0.030	0.97	774[b]	744[a]	771[b]	738[a]
ADG, g/d	RFI	0.011	1x10^{-8}	0.06	822[c]	765[b]	812[c]	707[a]
ADFI, g/d	CORT	0.64	0.011	0.45	2.21[a]	2.30[ab]	2.19[a]	2.36[b]
ADFI, g/d	RFI	3x10^{-13}	0.75	0.07	2.57[b]	2.65[b]	2.21[a]	2.10[a]
FCR	CORT	0.63	6x10^{-7}	0.58	2.85[a]	3.09[b]	2.84[a]	3.15[b]
FCR	RFI	8x10^{-11}	2x10^{-6}	0.38	3.12[c]	3.49[d]	2.72[a]	2.98[b]

[1] See text for the statistical models. Different letters indicate significant differences within row (*P*<0.05).
[2] See text for trait definitions.
[3] CORT lines: High line = High cortisol line, Low line = Low cortisol line; RFI lines: High line = less efficient; Low line = more efficient.

The Test diet reduced body weight and ADG, and increased FCR in the two sets of lines. There was no line x diet interaction for these traits in the cortisol lines, both responding very similarly to the dietary challenge. In the RFI lines, the ADG was more reduced by the Test diet in the low RFI pigs, resulting in tendency for a line x diet interaction. The ADFI increased with the Test diet in the cortisol lines (+135 g/d, *P*=0.01), as expected from the reduced energy content of the Test feed. However, this increase was lower than the 10% expected from the energy content change, in relation with increased gut fill and slower transit time due to the fibre content. This effect was not significant in the RFI lines, as the ADFI differences between Test and Control diets were in different directions in these lines (+78 g/d, high RFI pigs, *P*=0.28; -110 g/d, *P*=0.14, low RFI pigs), leading to a tendency for an interaction line x diet (*P*=0.07). The low RFI pigs, contrary to the other lines, seemed to be unable to increase their voluntary feed intake to face the lower energy and amino acid content of the Test diet. In addition, it has been shown previously that low RFI pigs have higher amino acid requirements per kg of feed (Gilbert *et al*., 2017), resulting in a larger impact on growth of a reduced amino acid content. In that respect, low RFI pigs were more sensitive to the dietary challenge than high RFI pigs.

Selection for plasma cortisol in the cortisol lines did neither affect the feed efficiency of the pigs, nor the ADG or ADFI when fed a conventional or the Test diet. This result does not confirm the initial hypothesis of impaired performances in high cortisol pigs. Analyses of larger datasets of growth showed similar responses under conventional feeding (Mormede *et al*., 2018), in accordance with the genetic correlation of 0.01±0.08 estimated between ADG and the selection criterion in these lines (Larzul *et al*., 2018). For FCR, no line x diet interaction was found, suggesting that the ability of the animals to cope with the dietary challenge in terms of efficiency was not affected by selection for plasma cortisol on one hand, or by selection for RFI on the other hand.

Table 23. Least square means[1] for line (High and Low) x diet (Control and Test) interaction on body and carcass composition[2] on the cortisol and RFI lines[3]

Trait	Line	Probability			High line		Low line	
		Line	Diet	Line x Diet	Control	Test	Control	Test
Backfat thickness, mm	CORT	2x10⁻⁴	0.27	0.76	15.69[a]	14.86[a]	17.63[b]	17.19[b]
Backfat thickness, mm	RFI	3x10⁻⁵	0.02	0.04	20.67[b]	20.51[b]	19.20[b]	16.35[a]
Weight-corrected backfat thickness, mm	CORT	9x10⁻⁵	0.61	0.61	15.78[a]	15.23[a]	17.43[b]	17.41[b]
Weight-corrected backfat thickness, mm	RFI	1x10⁻³	0.20	0.12	19.94[b]	20.14[b]	18.82[ab]	17.29[a]
Lean meat content, %	CORT	1x10⁻³	0.39	0.70	59.10[b]	58.87[b]	57.63[a]	57.05[a]
Lean meat content, %	RFI	0.66	0.31	0.57	56.57	56.75	56.00	56.63
Carcass yield, %	CORT	2x10⁻³	6x10⁻⁵	0.26	76.30[c]	74.74[ab]	75.03[b]	74.09[a]
Carcass yield, %	RFI	5x10⁻³	1x10⁻⁴	0.98	74.83[b]	73.60[a]	75.74[c]	74.56[b]

[1] See text for the statistical models. Different letters indicate significant differences within row ($P<0.05$).

[2] See text for trait definitions.

[3] CORT lines: High line = High cortisol line, Low line = Low cortisol line; RFI lines: High line = less efficient; Low line = more efficient.

Body and carcass composition. A cortisol line effect was found for all body and carcass composition traits (Table 23). The high cortisol line was leaner than the low cortisol line, as indicated by line differences for backfat thickness and lean meat content. This result is not consistent with the hypothesis of decreased cortisol levels in leaner pigs being associated with better production efficiency. In the RFI lines, the low RFI pigs were leaner in terms of backfat thickness than the high RFI pigs, as reported previously (Gilbert *et al.*, 2017). A diet effect was observed in the RFI lines but not in the cortisol lines. The significant reduction of backfat thickness observed in low RFI pigs fed the Test diet was no longer significant when the body weight of the pig was included in the model. This suggests a secondary effect of the reduced body weight of the low RFI animals rather than an increased leanness due to the feed restriction imposed by the dietary fibre content of the Test diet. Finally, line and diet effects were observed for both pairs of lines on carcass yield. The Test diet led to a reduced carcass yield that could be due to the increased development of the gut tract in response to the dietary fibre content of the feed (Montagne *et al.*, 2014). The high cortisol line had an increased carcass yield compared to the low cortisol line, certainly related to their higher leanness. The low RFI line had a higher carcass yield than the high RFI line, which was not observed by Montagne *et al.* (2014) at 10 weeks of age.

Conclusion. Selection for increased or decreased plasma cortisol levels after stimulation of the adrenal gland did not impact growth and feed intake traits, but had a significant impact on body composition and carcass yield, with improved traits in the high cortisol animals. The two lines had similar responses to the dietary challenge. On the other hand, lines selected for divergent RFI had different responses to the alternative diet, the more efficient line having a more reduced growth rate with the diet with lower energy and amino acid contents. In terms of FCR, this line remained more efficient. To conclude, selection for lower RFI reduced the ability of pigs to increase their voluntary feed intake when given fibrous diets, whereas selection for increased cortisol levels did not improve the pigs' robustness to the diet challenge.

3.2 Genetic background of feed efficiency

Using genomic tools gives access to the understanding of the molecular determinism of the traits. In this project, molecular tools were used at the genomic level and at the transcriptomic level. In both cases, two objectives were addressed: 1. To find biological markers that could be used on-farm as proxies of feed intake or feed efficiency to reduce the load of individual measurements in very large cohorts and improve genetic gain in the selected populations, 2. To identify the biological pathways used by the more efficient animals that could be used as tools to select and manage these animals. To get a better understanding of the responses and avoid selecting SNP that would be specific to some populations or breeding conditions, animals from different lines or bred in various conditions (e.g., in relation to feeding) were tested in the studies.

3.2.1 Whole genome studies using genotype to phenotype association

➢ Comparison of genomes of lines divergent for feed efficiency

Rationale. To improve the efficiency of feed utilization in laying hens, it is essential to understand the genetic basis of individual variation of feed efficiency. To achieve this, we used two brown egg layer (Rhode Island Red) lines from INRA that were divergently selected, at constant body weight and egg production for more than 40 generations for low (low RFI) and high (high RFI) residual feed intake (Figure 6A). The result of the selection is that strong differences between the two lines are found at other traits related to feed efficiency. For example, the high RFI chickens are characterised by higher feed intake (+89%) and lower adiposity than the low RFI chickens, increased diet-induced thermogenesis (+133%), and reduced liver lipogenesis. Considering the low abdominal fatness of high RFI birds, it was suggested that in this line fat was the substrate for heat production as supported by the total absence of abdominal fat in this line, an extremely rare condition in birds.

The aim of this study is to identify loci involved in the variation of RFI and the other associated traits. To do so, we integrated multi-omics data to characterize the genomes of the two divergent lines by DNA and RNA sequencing and to identify the regions under selection.

Experimental design. DNA-seq paired-end libraries were prepared using the TruSeq DNA Sample Preparation Kit (Illumina) and sequenced in 2x150bp paired end on a HiSeq3000 (Illumina) with four individuals per line, expecting a 25X depth. The read sets were then aligned against the Galgal5 genome reference after quality control, and the Genome Analysis Toolkit software (GATK) was used for realignment, recalibration, and variant calling. Variants were annotated by Variant effect Predictor package from Ensembl. Concerning the RNAseq data, the details of protocols are described in 3.2.2.
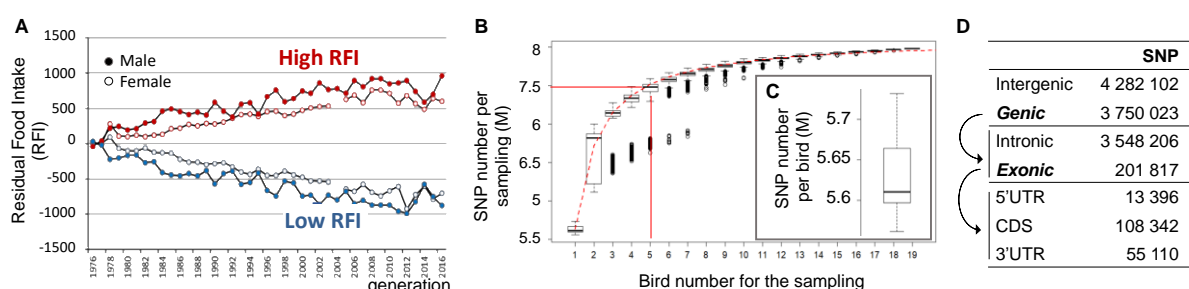
Main results

Figure 6. Characteristics of the SNP found within the genome of the high RFI and low RFI lines. A. Line responses to selection for RFI, B. Estimation of the number of SNP to be detected by resampling 1000 times combinations of 1 to 19 birds, C. Distribution of the number of SNP detected per bird, D. Distribution of the detected SNP depending on the genome annotation.

DNAseq analysis revealed more than 10M of variants taking the genome sequence as reference, with 86% of SNPs (8,911,615) and 14% of INDELs (1,440,977). After filtering on SNP quality criteria, 8M of SNPs were selected (8,032,125) with on average 5.6M SNP per individual (Figure 6C). Most of the SNPs present in the two genomes were captured by our analysis, as shown in Figure 6B indicating the number of unique SNP (Y-axis) obtained by resampling 1,000 times each combination from 1 to 19 birds (X-axis). Except for the chromosomes 11, 30, and 31, the SNP density was similar across all autosomes (macro or micro chromosomes), indicating a quite exhaustive SNP description. As expected, most of the SNPs were in the intergenic and intronic regions (98.7%; Figure 6D).

Concerning the genetic differences between the two high RFI and low RFI genomes, we found 661,325 SNP and 79,356 INDELS, within 7,720 and 5,356 genes, respectively, with contrasted frequencies between the high RFI and low RFI lines and at least one allele fixed in one line. Considering the small effective population size of the lines, genetic drift is strong and responsible for most the contrasted allele frequencies observed. The SNP list for further evaluation was thus restricted within 145 selection signatures previously identified to better target SNPs for which the variation resulted from selection (i.e., variation not compatible with a pure neutral evolution).

The next step will be to identify two types of candidate genes within these regions: i) genes harbouring a coding variant likely to affect the associated protein using the annotation of the 7.5 millions of variants detected with DNAseq data, ii) genes having a differential expression between the two lines (named cis-eQTL), thanks to a regulatory variant acting in cis. For the latter ones, we used the RNAseq data obtained from 150 transcriptomes of four different tissues chosen to be "*a priori*" related to feed efficiency to identify differentially expressed genes between lines (see second section 3.2.2 for the methods**.** In this analysis 3,784, 4,549, 6,658, and 2,470 genes were differentially expressed in liver, adipose tissue, blood and hypothalamus, respectively. We are currently selecting those that are within each selective sweep.

In parallel, this RNA-seq data allowed us to improve gene annotation of the chicken genome by generating an atlas of long non-coding genes. These genes are expected to be very important in the genotype-phenotype relationship thanks to regulatory roles of protein coding genes. More than 30,000 long non-coding genes were then identified in chicken, while Ensembl only lists more than 4,600 of them. As expected, some are differentially expressed between the two lines.

Main discussion points and conclusion. Most of the variants (SNPs and INDELs) present in high RFI and low RFI genomes are now described and a first set of about 150 selection signatures is to be characterised further. The large number of differentially expressed genes in four tissues indicate i) that selection for improved feed efficiency has not affected preferentially one tissue, but rather a large number of tissues and ii) that the causal mutations differentiating the two lines have important consequences detectable at the mRNA level. Further characterization of these regions will be conducted to identify candidate functional mutations based on this first exhaustive list.

➢ Association studies: associations between genomic variants and feed efficiency traits or its components (growth rate, feed intake, digestibility)

Association studies were conducted to detect SNPs associated with feed intake or feed efficiency that could be used to either directly select for better genotypes, or to understand the biological mechanisms involved in the variability of the traits. These studies were conducted in rabbits (i.e., first association studies reported in this species, after the development of the Affimetrix 200K SNP chip) for the first part, and in broilers to study genomic variants for digestive efficiency.

o Detecting associations on growth and feed efficiency traits in rabbits

Experimental design. Rabbits from a line selected for low RFI and an unselected control line (about 300 animals per line) were tested for individual growth, feed intake, and feed efficiency. Genotypes were available from the Affimetrix 200K SNP chip on 676 rabbits for 127,895 SNPs. A linear mixed model including the SNP effect as a regression on the allelic dose was applied to each trait. Candidate genes were searched for in the detected genomic regions increased by 1Mb upstream and downstream.

Main results and discussion. There was no significant association at the genome wide level (4×10^{-7}). However, considering chromosome wide levels (~4.5×10^{-4}), some significant signals could be detected for all traits (Figure 7). Moderate signals were detected on chromosome 5 for weight gain, chromosome 6 for feed intake, and chromosome 7 for FCR. A significant signal on chromosome 18 was detected for RFI. The peak comprised 20 significant SNPs from 47,518,182 to 48,092,199 base pairs (Table 24). In the same region, 1 SNP was significant for FCR at 47.6 Mb. In this region, about 15 genes are mapped, with implications in energy and protein metabolism, and cellular processes.
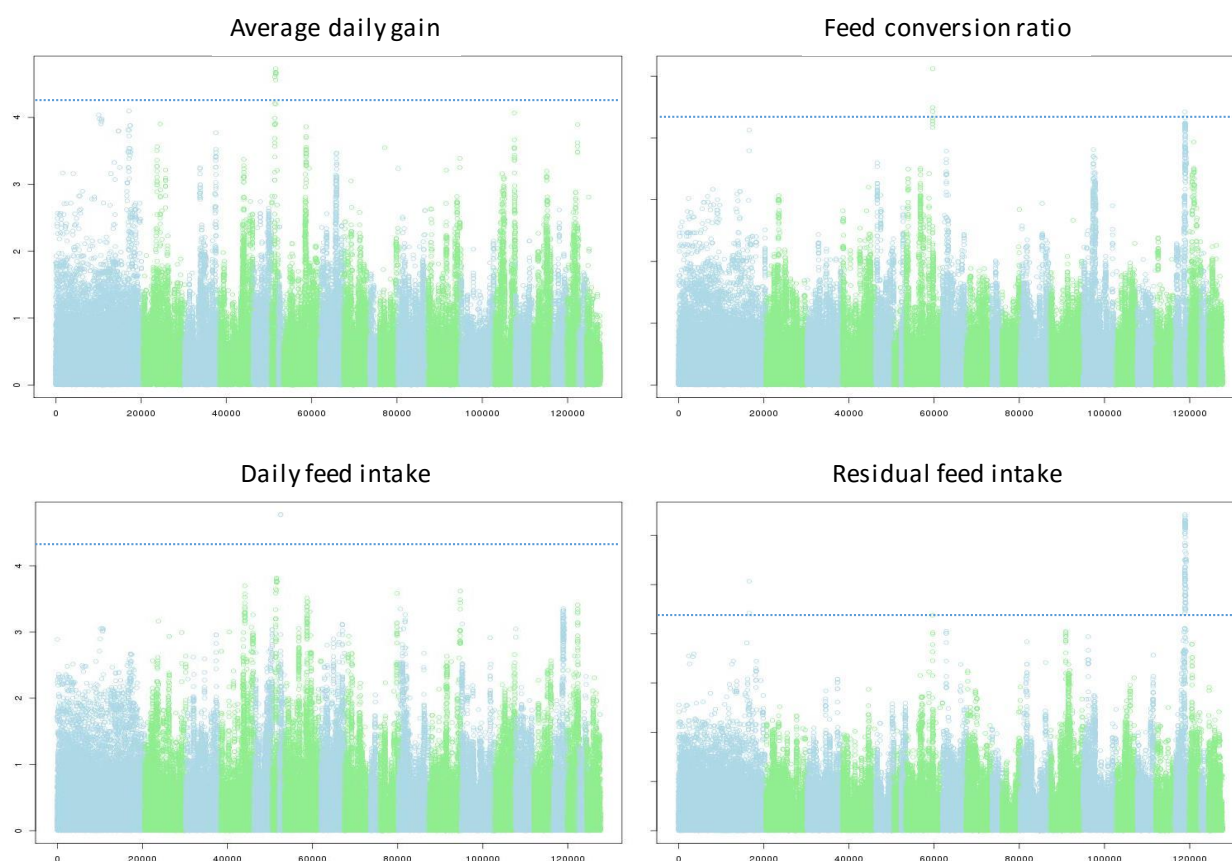
Figure 7. Manhattan plots (-log10(P-values)) for weight gain, feed intake, and feed efficiency traits in a rabbit population. The horizontal line indicates the chromosome wide threshold. The first block of SNP corresponds to the unlocated SNP.

Table 24. Detected QTL regions studies for growth, feed intake and feed efficiency traits and associated candidate genes.

Trait	Chromosome	Min-Max position	N SNP	Candidate genes
Weight gain	5	24.8 – 25.2	17	*PLA2G15-SLC7A6-PRMT7-SMPD3-ZFP90-CDH3-CDH1-HAS3-UTP4-SNTB2-NIP7-NFAT5NQO1-NOB1-WWP2-PSMD7*
Feed intake	6	37.8-37.9	2	*OTX2-ZP3-SSC4D-YWHAG-MDH2-STYXL1-POlow RFIRHBDD2-EPHB4-ZAN-EPO-GNB2-GIGYF1-FBXO24-POP7-ACTL6B-TFR2-SAP25-LRCH4-AGFG2-NYAP1-TSC22D4-PPPR35-MEPCE-ZCWPW1-STAG3*
RFI	18	47.5-48.0	20	*HPSE2-CNNM1-GOT1-ABCC2-ANTPD7-COX15-PKD2L1-DNMPB-CPN1-ERLIN1-CHUK-BLOC1S2-WINT8B-SEC31B- H1F1AN-PAX2*
FCR	7	12.4-12.5	3	*CCDC192-SLC12A-FBN2-SLC27A6-ISOC1-ADAMTS19-MINAR2*
FCR	18	47.6	1	

Conclusion. Associations could be detected for all traits, especially for RFI, which was the selection criterion to establish the differences between the lines. Among all the genes located in these regions, no clear candidate could be identified and further studies will be needed to identify causative variants.

o   Detecting associations on feeding traits recorded at the cage level

Experimental design. A total of 446 samples from rabbits with performance were available (230 under *ad libitum* feeding and 206 under restricted feeding) for genotyping. Growth rates were recorded for individual animals, but feed intake was recorded at the cage level. Similarly, feed restriction was applied at cage level. Genotypes from the Affimetrix 200K SNP chip were obtained and, after quality control, a total of 114,604 SNP were retained for the association studies. Two different statistical methods were used:

1. A linear mixed model including the SNP effect as a regression on the allelic dose (Pérez-Enciso, and Misztal, 2011). This model was applied to individually recorded traits, growth under *ad libitum* feeding and under feed restriction.
2. A bivariate linear mixed model including the SNP effect as a regression on the allelic dose applied jointly on individual growth and cage feed intake (Legarra and Vitezica, 2015) to resolve the difficulty of performing a genome-wide association study on cage records. This analysis allows fitting records of animals without genotypes. Thus, all records from cages having genotyped animals were considered, not only the 446 with genotypes. Because it is computationally demanding, this analysis was applied to 1/10 of the SNPs, representative of linkage blocks defined with the PLINK software.
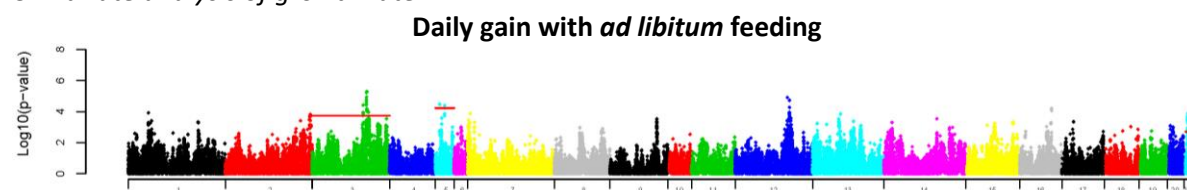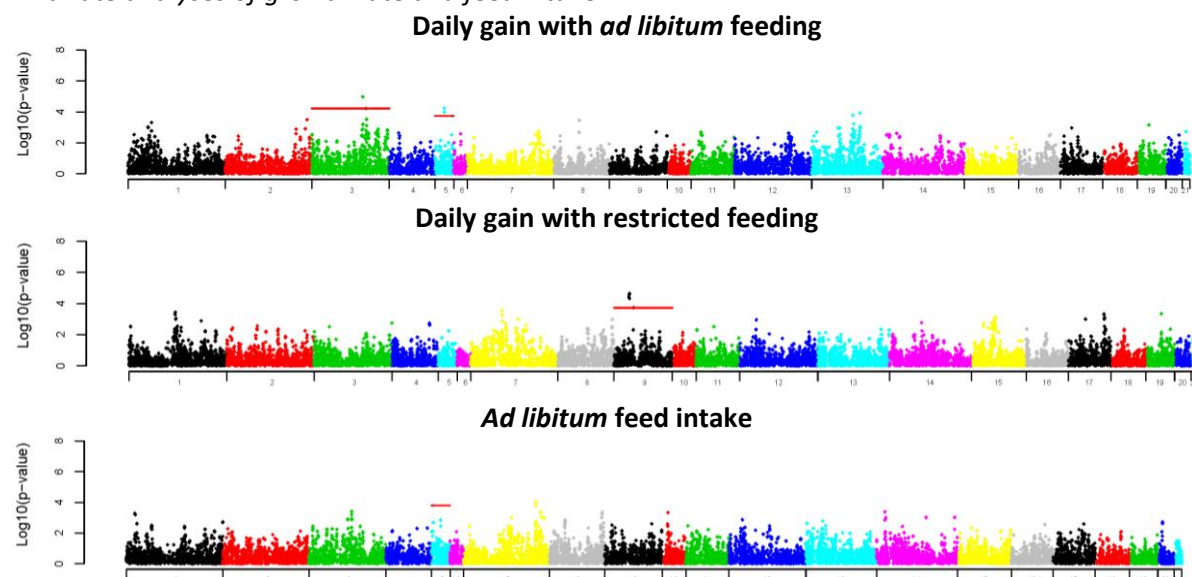
Univariate analysis of growth rate

Daily gain with *ad libitum* feeding

Bivariate analyses of growth rate and feed intake

Daily gain with *ad libitum* feeding

Daily gain with restricted feeding

***Ad libitum* feed intake**

Figure 8. Manhattan plots for univariate and bivariate association studies for growth and feed intake traits under ad libitum or restricted feeding. Horizontal red lines indicate the chromosome-wide significance level, only presented for chromosomes in which this threshold was reached.

Main results and discussion. No association was significant at the genome-wide level for the studied traits, regardless of the method employed. Chromosome-wide significant associations for growth under *ad libitum* feeding were detected in chromosomes 3, 5, and 21 for the first univariate model (Figure 8). With the bivariate approach, the same genomic regions were detected for growth under *ad libitum* feeding in chromosomes 3 and 5, but also one for growth under feed restriction on chromosome 9, and for feed intake under *ad libitum* feeding on chromosome 5. Figure 8 presents the Manhattan plots for these association studies for traits showing some significant chromosome-wide associations. Table 25 describes these QTL regions, including the candidate genes found in their neighbourhood (1Mb beyond the start and the end of each QTL region).

Conclusions. Univariate analyses identified QTLs for ADG of animals fed *ad libitum* while bivariate analyses identified QTLs for all traits studied. In spite of the limited power of the design, five QTL regions were detected for ADG of animals fed *ad libitum,* one QTL region was declared for ADG of animals fed under restriction and one QTL region was declared for feed intake. Ten candidate genes related to feed efficiency and its components (i.e., growth and feed intake) were identified in these QTL regions.

Table 25. Detected QTL regions for growth (ADG) and feed intake (FI) traits under *ad libitum* or restricted feeding, with univariate and bivariate analyses, and associated candidate genes in ±1Mb around the maximum SNP.

Trait	Chromosome	Min-Max position –Mb)	N significant SNPs (/total SNPs in the linkage disequilibrium block)	Minor Allele Frequency	Candidate genes
Univariate analysis					
ADG ad libitum	3	101-114	27	0.23	*NDUFAF6*
ADG ad libitum	5	8-10	1	0.11	*FTO*
ADG ad libitum	5	18-20	2	0.29	--
ADG ad libitum	21	6-9	25	0.06	*ATXN2, ACAD10, TRAFD1, PTPN11*
Bivariate analysis					
ADG ad libitum	3	101-110	2/17	0.38	*NDUFAF6*
ADG ad libitum	5	18-20	2/10	0.19	--
ADG ad libitum	5	33-35	1/2	0.17	*DYNLRB2*
ADG restricted	9	29-40	6/35	0.07	*FEZF2*
Feed intake ad libitum	5	2.8-4.9	1/5	0.37	*CEBPA, KCTD15*

Compared to the rabbit study presented in the previous section, no QTL region was found in common. Preliminary joint analyses of these genotypes showed a different genetic basis for the populations of the two designs (Figure 9), so no joint association study is envisaged.
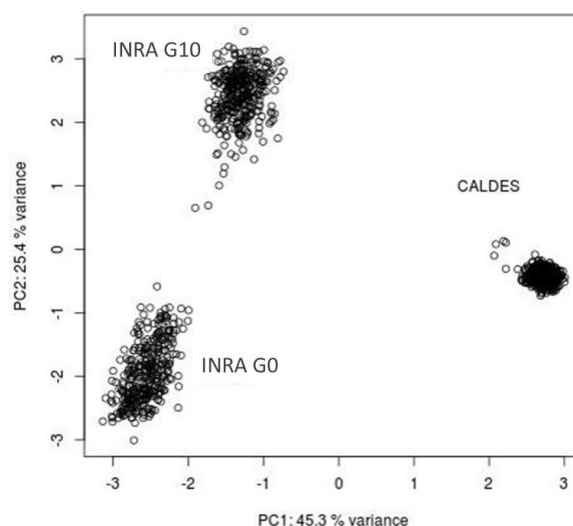

Figure 9. Principal Component Analysis on INRA rabbit lines (G0=Control line, G10 = selected line) and IRTA Caldes rabbit population.

    o   Associations studies on digestibility traits in broilers

Rationale of the work. For reasons of sustainability, the composition of chicken diets will evolve in the future to incorporate different feeding resources. These new diets will be more diverse and

nutritionally less adequate than the traditional cereal-soybean diet. Birds will thus have to have a good capacity to adapt to these new feedstuffs to maintain their growth and feed efficiency, without decreasing meat quality and carcass composition. To propose selection strategies to adapt chickens to this new context, we searched for the genetic basis of digestive efficiency in chickens, which is an essential component of feed efficiency.

Experimental design. The experiment relied on 192 broilers from the 8th generation of an advanced intercross line between two divergent lines of broilers selected for their high or low digestive efficiency. The criterion of selection for digestive efficiency was the apparent metabolisable energy (AMEn) content at 3 weeks of age, using a wheat-based diet difficult to digest. Before the cross started, the AMEn difference between the lines was between around 30-40%.

Birds were reared on the floor from hatch to 11 days and then transferred to individual cages for digestive efficiency measurements. Animals were fed a wheat-based diet containing 55% of Rialto wheat, a viscous and hard variety wheat. At 3 weeks, a balance trial was performed, using the method of total collection of faeces of Bourdillon *et al*. (1990). After freeze-drying, faeces were analysed by NIRS to obtain AMEn, and coefficients of digestive use of dry matter, starch, nitrogen, and lipids (Bastianelli *et al*., 2010). At the end of balance trial, blood samples were taken and genotyped with the 540K SNP Affymetrix® Axiom® chip. After quality control, 353,888 SNP were retained for analysis, located on chromosomes 1 to 28 and 33. Phenotypic data were pre-corrected for significant fixed effects. Then, a univariate linear mixed model (polygenic effect, SNP effect) was used to detect associations between SNP and phenotypes. A Bonferroni correction was applied at the genome level to account for multiple testing.

Main results. A total of 12 SNPs were genome-wide significant (Bonferroni adjusted *P*-value<0.05) for AMEn, coefficients of digestive use of dry matter, starch, and nitrogen. None was significant for the digestibility of lipids. Figures 10 and 11 show the resulting Manhattan plots obtained for AMEn and the digestibility coefficient of starch.
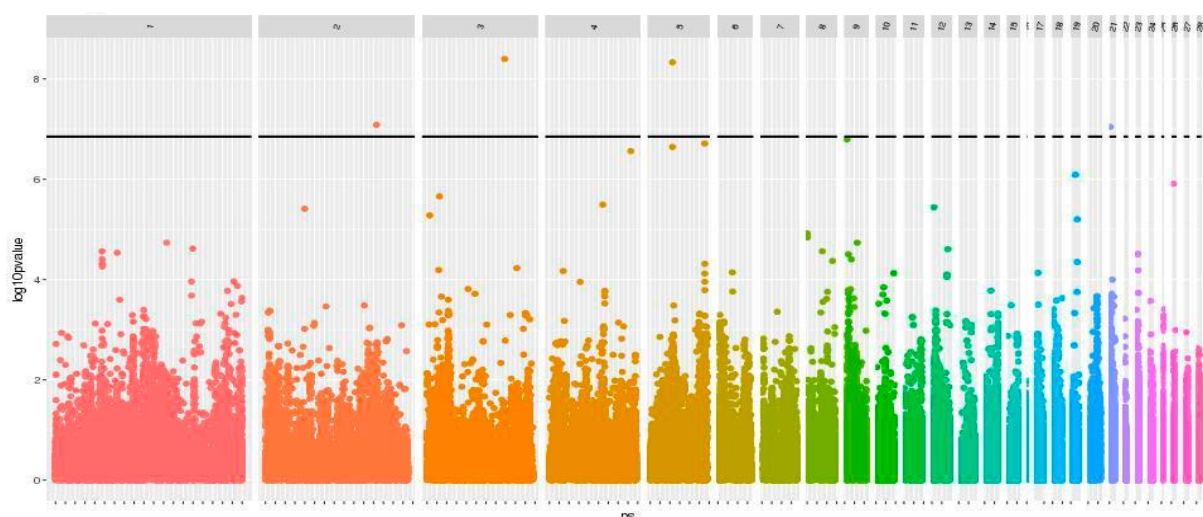

Figure 10. Manhattan plot of the association study for AMEn. The horizontal line indicates the genome-wide threshold at 5%.
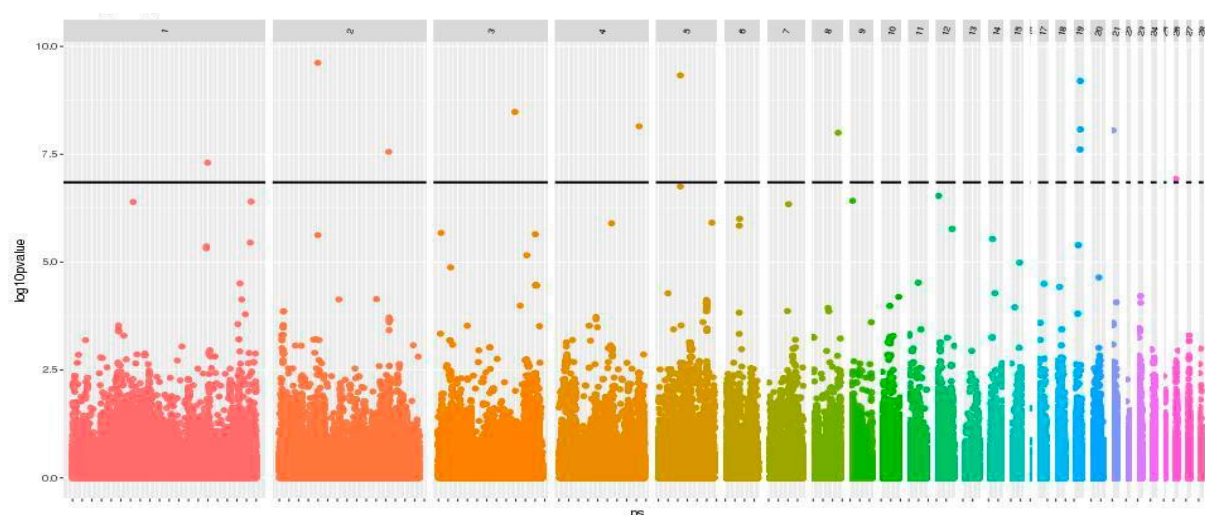
Figure 11. Manhattan plot of the association study for the digestibility coefficient of starch. The horizontal line indicates the genome-wide threshold at 5%.

Among the 12 genome-wide significant SNPs (Table 26), seven were located in intronic regions of known genes and one was in the regulatory region of C1ORF174. These genes are known to be involved in intestinal morphology and development (i.e., ZIC2, ANO10, and F2), immune system functions (i.e., SULF1, CUD109, and F2), and regulation of energy balance (i.e., FGGY andPM20D1). All these SNP were significant for at least two digestive traits, suggesting general mechanisms involved in digestion rather than specific mechanisms related to fractions of the ration.

Conclusion. Some SNPs were detected as being involved in the determinism of the digestive efficiency in broilers, affecting two to four traits. Some are located in genes annotated with intestine-related functions. Further analyses are needed to evaluate their segregation and effects in different broiler populations, and to identify the underlying biological mechanisms. The underlying mutations could be used in selection programs.

Table 26. Genes associated with the 5% genome-wide significant SNPs.

Chromosome	SNP	Trait	Within Gene/Intergenic
1	144 284 848	Digestibility (DM, starch, nitrogen)	*ZIC2*
2	41 039 328	Digestibility (DM, starch, nitrogen)	*ANO10*
2	116 692 708	AMEn, Digestibility (DM, starch, nitrogen)	*SULF1*
3	81 690 202	AMEn, Digestibility (DM, starch, nitrogen)	*CD109*
4	85 583 360	Digestibility (DM, starch, nitrogen)	Intergenic
5	23 444 968	AMEn, Digestibility (DM, starch, nitrogen)	*F2*
8	26 671 543	Digestibility (starch, nitrogen)	*FGGY*
19	6 390 258	Digestibility (starch, nitrogen)	Intergenic
19	6 677 286	Digestibility (starch, nitrogen)	Intergenic
19	6 764 624	Digestibility (starch, nitrogen)	Intergenic
21	897 100	AMEn, Digestibility (starch, nitrogen)	1000 bp before *C1ORF174*
26	2 317 884	Digestibility (starch, nitrogen)	*PM20D1*

3.2.2   Expression studies

➢   Identifying genes for which the expression is affected by selection for feed efficiency and by breeding conditions in layers

Rationale. The divergent lines selected for low (low RFI) and high (high RFI) residual feed intake (Figure 6A) were submitted to a main breeding stressor: a low-energy diet with an energy content 15% below a conventional diet. In previous projects, we have shown that birds can cope well with changes in dietary energy content. The egg mass was maintained whereas the low energy content of the diet was compensated by an increased feed intake and decreased body fat storage. Interaction between diet and genotype was not found for any trait. To better understand how the two lines cope with this dietary challenge, the transcriptome of the four main tissues involved in energy homeostasis (i.e., liver, adipose tissue, hypothalamus, and whole blood) was examined.

Experimental design. A total of 100 female birds equally distributed among the two lines that were given either a control diet (2900 kcal; ~70 birds) or a suboptimal low-energy diet (LE: 2450kcal; ~30 birds). All birds from the LE condition were fed *ad libitum* with a standard diet until 17 weeks of age after which the bird received their respective diet until 31 weeks of age. A subset of 32 animals (8 per combination of line and diet) were slaughtered for tissue sampling. The RNA was extracted and the polyA+ RNA from the four tissues was sequenced (90 M stranded and paired-end reads per sample). The RNA sequences were then aligned on the GalGal5 genome using STAR software and the expression calculation were performed with RSEM software usingV94 Ensembl annotation that contains 24,881 genes, mainly protein-coding genes. Differential analyses for lines and two dietary treatments were performed with the EdgeR R package; the interaction line x diet was systematically included in the statistical analysis. Genes were considered as differentially expressed (DE) for a *P*-value corrected for multiple testing false discovery rate of less than 0.05.

Main results. Most of the 24,881 genes were expressed in at least one of the four tissues. Surprisingly, the diet had a large effect on the hypothalamic and blood transcriptome, while the hepatic and adipose tissue transcriptome were almost unaffected (Figure 12A). Very small numbers of significant interactions were observed (less than five genes per tissue). The KEGG enriched pathways analysis of the set of differentially expressed genes in the hypothalamus highlighted a mechanism consistent with increased feed intake in the low-energy diet (Figure 12B). In blood, the interpretation of the eight KEGG enriched pathways remained unclear, with two pathways associated to the ribosomes for the overexpressed genes in response to the low-energy diet and six pathways associated to the amino acid, sugar, and steroid metabolism for the overexpressed genes for birds receiving the low-energy diet.

A

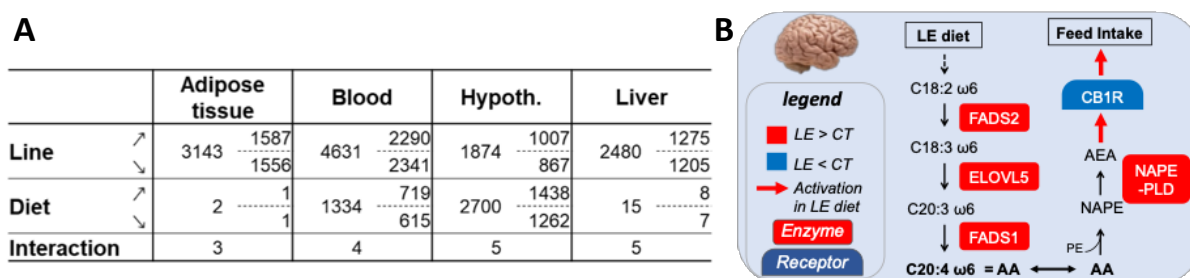|		Adipose tissue		Blood		Hypoth.		Liver	
Line	↗	3143	1587	4631	2290	1874	1007	2480	1275
	↘		1556		2341		867		1205
Diet	↗	2	1	1334	719	2700	1438	15	8
	↘		1		615		1262		7
Interaction		3		4		5		5	

B

Figure 12. Impact of the diet and line on transcriptomes. A. Numbers of genes differentially expressed for the two factors in four tissues. B. Impact of the low-energy (LE) diet on the endocannabinoid system in the hypothalamus.

Main discussion points and conclusion. We did not observe an interaction between diet and genotype. Moreover, we observed a tissue-specific response with a limited role of metabolically active tissues, such as the liver and adipose tissue compared to blood and the hypothalamus, at least at the transcriptome level. In the hypothalamus, a pathway related to the endocannabinoid system was identified, which could explain the increase in feed intake of animals fed the low-energy diet.

➤ Expression studies divergent lines in pigs, biological relationships between feed efficiency and responses to stressors

Rationale. Pigs from lines divergently selected for RFI and from lines with different plasma cortisol levels one hour after an ACTH injection were tested for their response to the ACTH injection as described in section 3.1.3. During the ACTH test, blood samples were collected before the ACTH injection, and one hour and four hours after the injection, with the objective to identify genes differentially expressed within the two RFI lines and within the cortisol lines, and to compare their biological responses to the stimulation of the HPA axis to understand the biological mechanisms behind better feed efficiency and higher HPA activity.

Experimental design. Among the four batches of seven pigs of tested per line, the batch with the response to ACTH injection closest to that of the full dataset was selected to extract blood RNA. Based on earlier analyses, 88 probes of genes involved in the cortisol general metabolism were retained to build a Fluidigm chip (Sautron *et al*., 2015) and to measure the differential responses between lines and between times of measurements. After normalization and quality control, five genes were excluded from further analyses, and one sample per line was eliminated due to a high number of outliers. The remaining dataset contained 68 samples with a maximum of eight missing measurements across genes and times. To evaluate the effect of line and time within each protocol (RFI and cortisol), linear mixed models were applied to each gene expression independently, including the fixed effects of line and time and their interaction, and a random effect of the pig to account for the repetition across times. Genes showing a false discovery rate greater than 0.01 were considered as significant.

To describe the response to the ACTH injection in a more comprehensive manner, principal component analyses were applied to the expression of the genes, and partial correlations with blood measurements during the test (i.e., IGF-I, urea, free fatty acids, glucose and cortisol) were estimated.
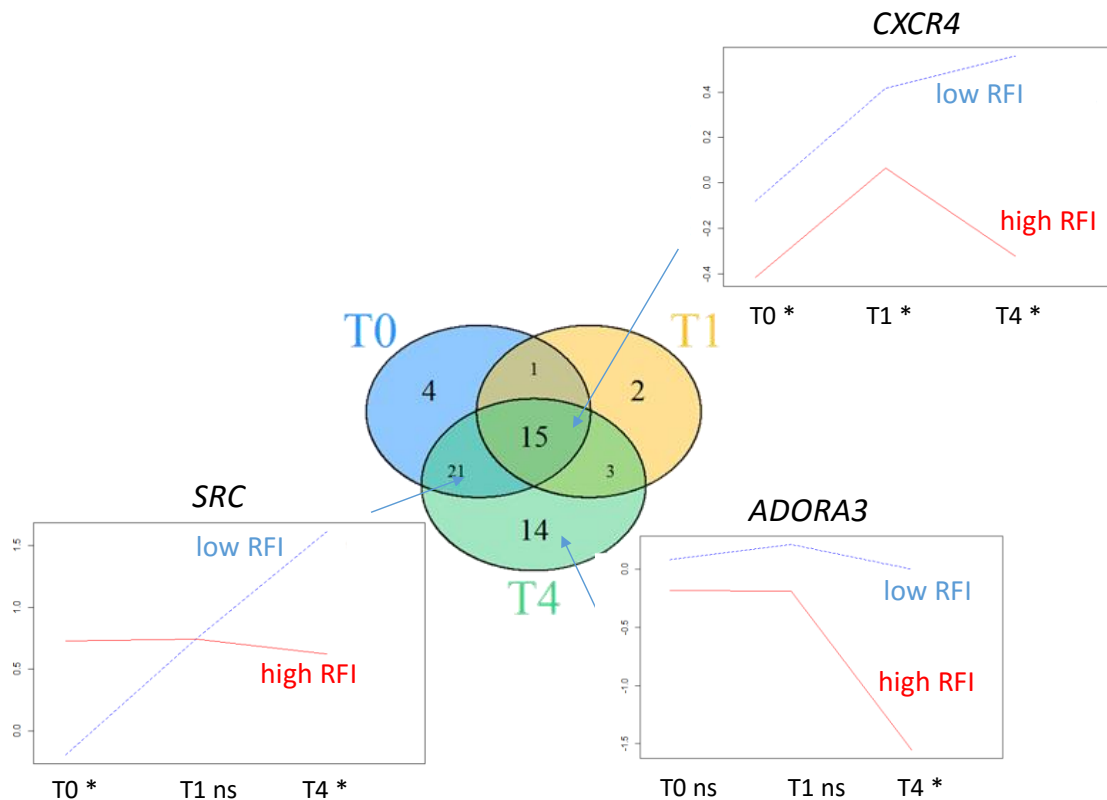
Feed-a-Gene

*Figure 13. Venn diagram of the differentially expressed genes between RFI lines at each time of sampling (T0 = before the ACTH injection, T1 = one hour after ACTH injection, T4 = 4 hours after ACTH injection). The graphs show examples of expression responses for three of the four genes differentially expressed between times and lines (significant interaction *: P<0.05).*

Main results. Altogether, 64 probes were differentially expressed in the comparison of the RFI lines, including 57 with significant line effect, 50 with significant time effect, and 4 significant for the interaction between line and time (i.e., SRC, CXCR4, ADORA3, and SSH1). Only SRC showed a significant difference before injection. Among the genes affected by time, 42 were differentially expressed between times in the low RFI line and 30 in the high RFI line. Considering the three times separately, at least 21 genes were differentially expressed (1 hour after injection) and at most 53 genes were differentially expressed (4 hours after injection). This indicates a significant difference in metabolism between the RFI lines related to the function of the HPA-axis at the basal stage, and even more so 4 hours after injection. This maximum difference of the expression measurements 4 hours after injection has been reported in a previous study (Sautron *et al.*, 2015) on the cortisol lines. Among the genes differentially expressed between lines at each time, 15 are common to the 3 time points, and 21 are common to the measurements before infection and 4 hours after injection (Figure 13).
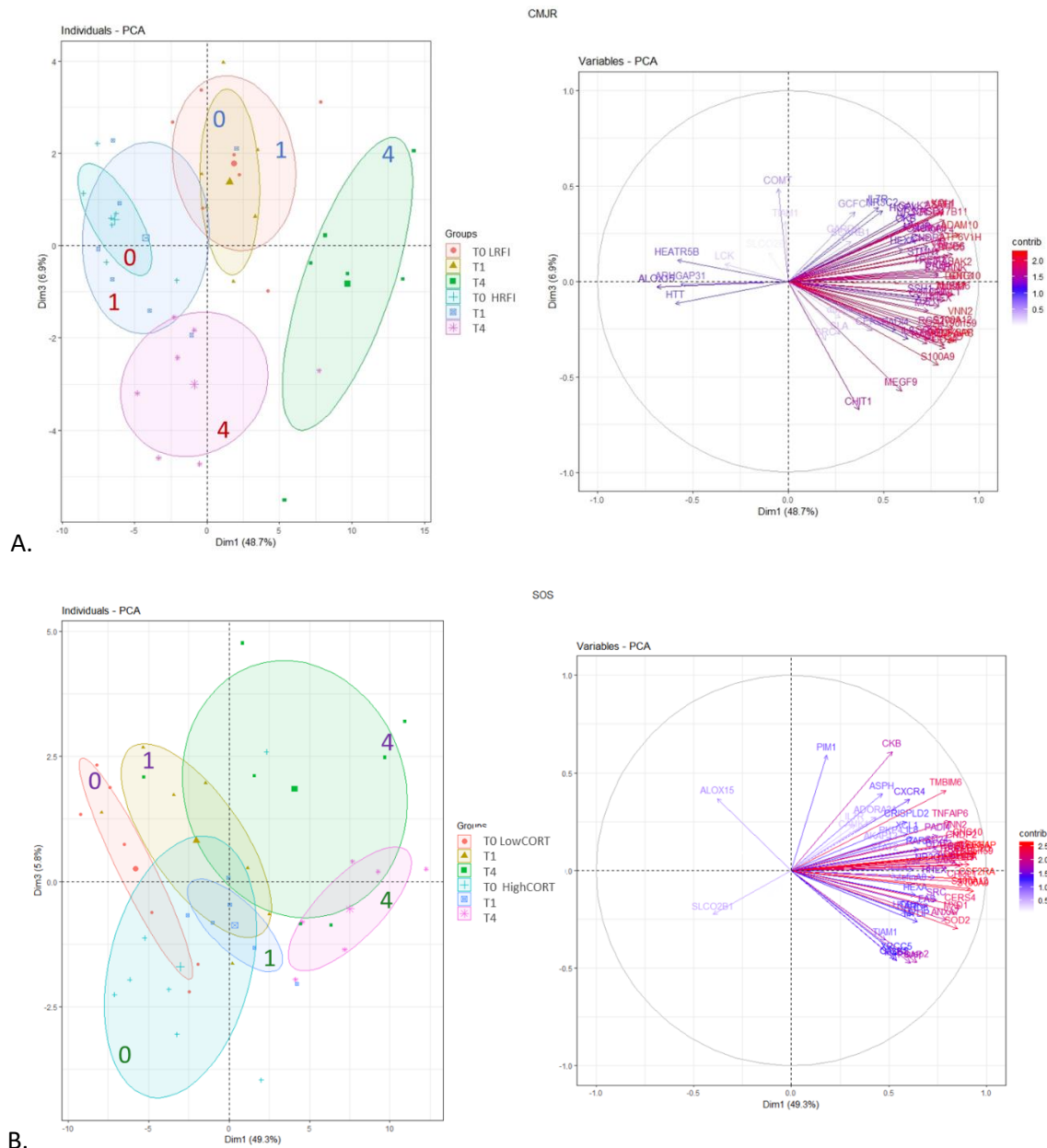
Figure 14. Principal components analysis of the RFI lines samples (A) and the cortisol lines (B) at three time points, and projection of the variables (genes) in the resulting space.

The comparison of the results in the cortisol lines show only two genes differentially expressed between the lines, with one showing an interaction with time (ADORA2A). No genes differed when considering the times independently. The 58 genes differentially expressed relative to time confirm that the test was properly performed and that animals responded. Based on this analysis, the biology of the cortisol lines responds in a more homogeneous manner than the RFI lines.

The principal component analysis applied to RFI lines only separated the two lines according to a diagonal (Figure 14A). The first axis (49% of the variance) essentially discriminated the 0 and 1 hour measurements in the high RFI line from the 4 hours measurements in the low RFI line, whereas the other axis separated discriminated the 0 and 1 hour measurements in the low RFI line from the 4 hours measurements in the high RFI line (7% of the total variance). The projection of the variables showed

that most of the gene expressions were correlated to the first axis, which confirms that the 4 hours time point is the driver of the variability in this dataset.

A similar pattern was observed on the cortisol lines (Figure 14B), but the two lines converged to the same direction of the first axis, consistently with the linear analyses showing less difference at that time point. The genes defining the axes were very similar in the two sets of lines, which is interpreted as similar responses in all lines, but with contrasted magnitudes for the RFI and the cortisol lines.

To conclude, among the genes differentially expressed with time in each line, 23 were found in the four lines (Figure 15). These genes can be considered as representing the basal pathways recruited during the response to the ACTH injection. Only nine additional genes are common to the RFI lines, and four to the CORTISOL lines. The most interesting pathways will be those found only in the low RFI line (six genes), or shared between the efficient and responsive pigs (four genes).
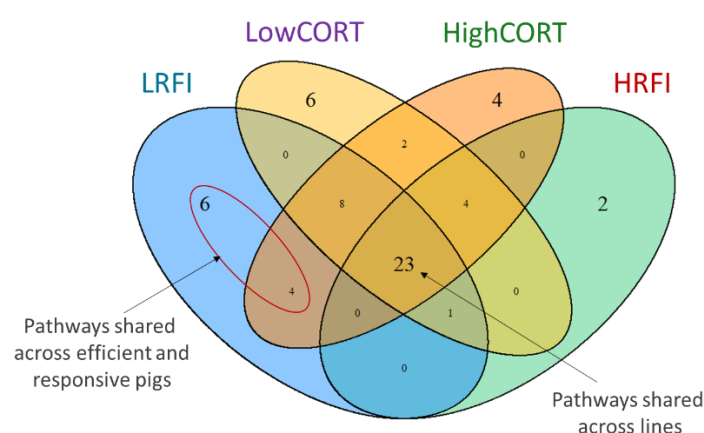

Figure 15. Venn diagram of the differentially expressed genes between time points in all lines.

From the partial correlations study, it seems that all the main pathways are implicated in the response to the injection in the RFI lines, whereas it is essentially the metabolism directly related to the cortisol that is related to the differentially expressed genes levels in the cortisol lines (Figure 16). Compared to the previous results in these lines (see deliverable D2.6 for more details), this is new and potentially specific to the response to the injection.
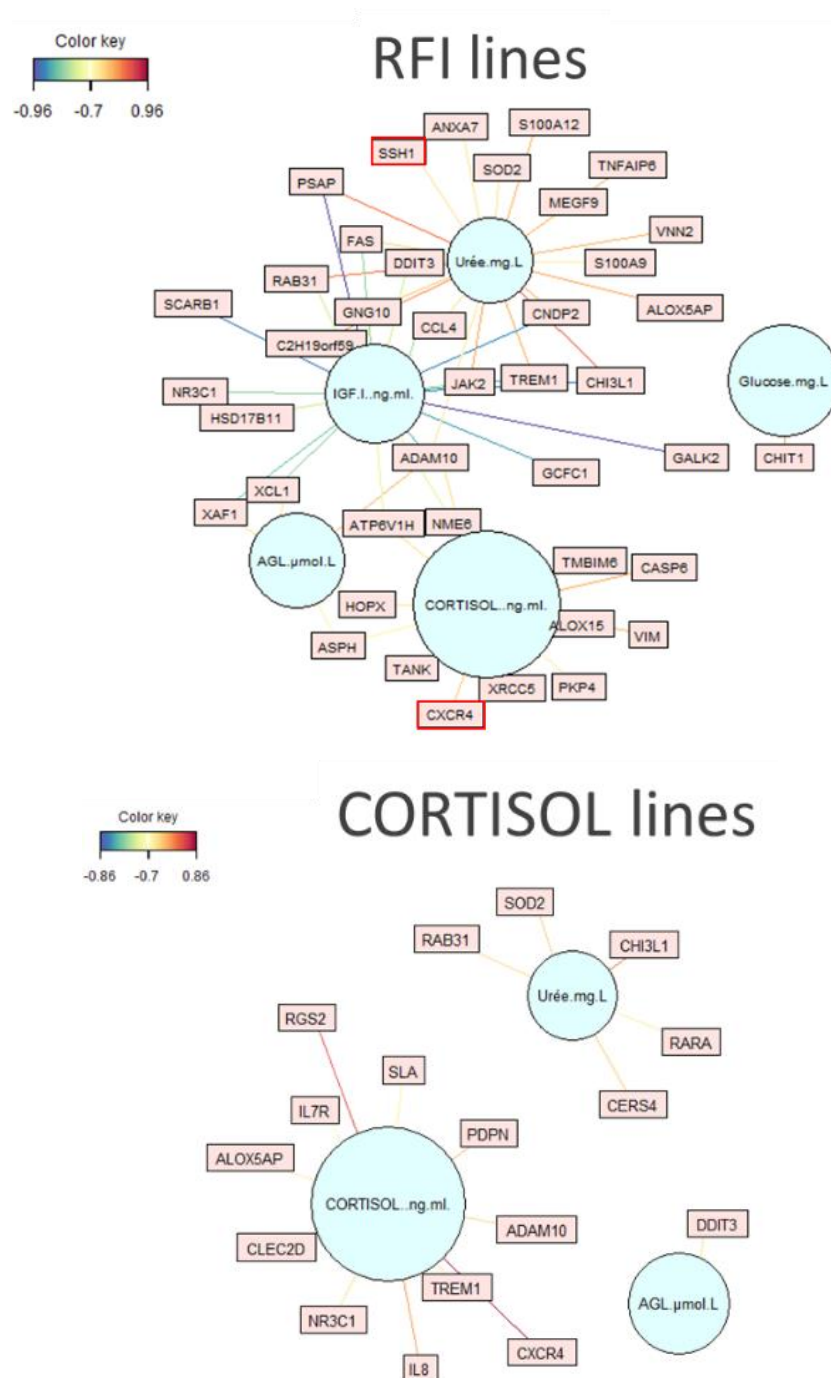
Figure 16. Partial correlations with the blood measurements of indicators of energy, cortisol, amino acid metabolism during the test. Red squares indicate genes that were differentially expressed between line and time.

Main discussion points and conclusion. The main driver of the differences reported for this test is related to the measurements 4 hours after injection (i.e., to the mechanisms recruited in response to the injection). It is notable that the measurements of cortisol in plasma at that stage indicate levels very similar to the basal stage, except for the low RFI line, whereas the expression data show that the response at the metabolic level is not completed. This difference is more important in the RFI lines, and seems related to pathways involved in amino acid metabolism. These results open new perspectives to understand the relationships between responses to stress and feed efficiency. Indeed,

Feed-a-Gene

most of the differences between the RFI lines reported until now have been related to the energy and immunity metabolism (see deliverable D2.6), but insights in their responses to different stressors have shown different response dynamics that remained to understand.

4. Conclusions

Thanks to a combination of different genetic approaches and new measurements, novel traits were found for direct measurements of feed efficiency, but also for the components of feed efficiency and for biomarkers for feed efficiency. All the monogastric species studied in Feed-a-Gene were covered by our studies. Most of these traits will need further validations, either in other populations or in larger datasets for further implementations in breeding schemes. Some will be taken on board in the evaluation of new breeding strategies for feed efficiency in the last steps of the genetic studies to be run in the Feed-a-Gene project. In conclusion, the following traits indicative for feed efficiency can be considered in selection and breeding:

- Traits adopted for evaluation in breeding strategies scenarios in Task 5.4:
    - Digestibility measurements (pigs)
    - Biomarkers for digestion (broilers)
    - Number of head hits received (pigs)
    - Eating rate and occupation time at the feeder (pigs, and potentially rabbits and broilers)
    - Blom's rank score from feeding behaviour traits (pigs)
    - Blood counts (pigs)
- Traits to be consolidated before integration in breeding programs:
    - Measures of feed efficiency in reproductive pig females, during gestation and lactation
    - Leg lesions (pigs)
    - Hair cortisol at the end of the fattening period (pigs)
    - Genomic and biological pathways associated with feed efficiency (pigs, rabbits, broilers, and layers)
- Traits adopted for demonstration in Task 5.5
    - Individual measures of feed intake (rabbits)

5. Annexes

5.1   Composition of experiment diets for the pig digestibility trial

	Conventional diet	High fibre diet
Ingredients, %		
Wheat	42.1	38
Mais	25	-
Barley	10	16.9
Rapeseed meal	6	6
Soyabean meal	10.5	5.4
Sunflower meal	3	3
Wheat bran	-	15
Soyabean hulls	-	8
Sugar beet pulp	-	5
Calcium carbonate	1.4	1.12
Bicalcium phosphate	0.49	0.29
Sodium chloride	0.4	0.4
Vitamins and mineral premix	0.4	0.4
L-Lysine HCl	0.44	0.35
DL Methionine	0.09	0.025
L-Threonine	0.13	0.011
L-Tryptophan	0.02	-
Chemical composition. %		
Dry matter	87.2	87.6
Ash	4.9	5.4
Crude protein	15.7	15.0
Crude fibre	4.1	8.4
Ether extract	2.1	1.9
NDF	13.9	24
ADF	5.3	10.6
ADL	1.6	2.2
Net energy (MJ/kg)	9.6	8.2

5.2 References

Bergsma R., Kanis E., Verstegen M.W.A., Knol E.F. (2008). Genetic parameters and predicted selection results for maternal traits related to lactation efficiency in sows. Journal of Animal Science 86: 1067-1080.

Blum Y., Monziols M., Causeur D., Daumas G. (2014). Recalibrage de la principale méthode de classement des carcasses de porcs en France. Journées de la Recherche Porcine 46: 39-44.

Casal N., Manteca X., Escribano D., Cerón J.J., Fàbrega E. (2017). Effect of environmental enrichment and herbal compound supplementation on physiological stress indicators (chromogranin A, cortisol and tumour necrosis factolow RFIα) in growing pigs. Animal 11: 1228-1236.

Do D.N., Strathe A.B., Jensen J., Mark T., Kadarmideen H. (2013). Genetic parameters for different measures of feed efficiency and related traits in boars of three pigs breeds. Journal of Animal Science 91: 4069-4079.

Fairbanks L.A., Jorgensen M.J., Bailey J.N., Breidenthal S.E., Grzywa R., Laudenslage M.L. (2011). Heritability and genetic correlation of hair cortisol in vervet monkeys in low and higher stress environments. Psychoneuroendocrinology 36: 1201-1208.

Ibáñez-Escriche N., Varona L., Magallón E., Noguera J.L. (2014). Crossbreeding effects on pig growth and carcass traits from two Iberian strains. Animal 8: 1569-1576.

Jégou M., Gondret F., Vincent A., Tréfeu C., Gilbert H., Louveau I. (2016). Whole blood transcriptomics is relevant to identify molecular changes in response to genetic selection for feed efficiency and nutritional status in the pig. PLoS ONE 11: e0146550.

Knap, P.W. (2009). Allocation of resources to maintenance. In: Resource allocation theory applied to farm animal production (W.M. Rauw, ed.). CABI Publishing, Wallingford, UK, p 210–229.

Gilbert, H., Billon Y., Brossard L., Faure J., Gatellier P., Gondret F., Labussière E., Lebret B., Lefaucheur L., Le Floch N., Louveau I., Merlot E., Meunier-Salaün M.C., Montagne L., Mormede P., Renaudeau D., Riquet J., Rogel-Gaillard C., van Milgen J., Vincent A., Noblet J. (2017). Review: divergent selection for residual feed intake in the growing pig. Animal 11:1427-1439.

Gilbert H., Bidanel J.P., Billon Y., Lagant H., Guillouet P., Sellier P., Noblet J., Hermesch S. (2012). Correlated responses in sow appetite, residual feed intake, body composition, and reproduction after divergent selection for residual feed intake in the growing pig. Journal of Animal Science 90: 1097-1108.

Larzul C., Terenina E., Foury A., Billon Y., Louveau I., Merlot E., Mormede P. (2015). The cortisol response to ACTH in pigs, heritability and influence of corticosteroid-binding globulin. Animal 9: 1929-1934.

Larzul C., Terenina E., Billon Y., Mormede P. (2018). Genetic determinism of cortisol levels in pigs. In: Proceedings of the World Congress on Genetics Applied to Livestock Production, vol. Species - Porcine 2, p. 214. February 11-16, 2018, Auckland, New Zealand. Massey University.

Legarra A., Vitezica Z. (2015). Genetic evaluation with major genes and polygenic inheritance when some animals are not genotyped using gene content multiple-trait BLUP. Genetics, Selection, Evolution 47: 89.

Lu D., Jiao S., Tiezzi F., Knaer M., Huang Y., Gray K.A., Maltecca C. (2017). The relationship between different measures of feed efficiency and feeding behavior traits in Duroc pigs. Journal of Animal Science 95: 3370-3380.

Montagne L., Loisel F., Le Naou T., Gondret F., Gilbert F., Le Gall L. (2014). Difference in short-term responses to a high-fibre diet in pigs divergently selected for residual feed intake. Journal of Animal Science 92: 1512-1523.

Mormede P., Terenina E. (2012). Molecular genetics of the adrenocortical axis and breeding for robustness. Domestic. Animal Endocrinology 43: 116-131.

Mormede P., Terenina E., Billon Y., Ferchaud S., Gress L., Remignon H., Manse H., Larzul C. (2018). In: Proceedings of the World Congress on Genetics Applied to Livestock Production, Volume Electronic

Poster Session - Species - Porcine 2, p. 229. February 11-16, 2018, Auckland, New Zealand. Massey University.

Noguera J.L., Ibáñez-Escriche N., Magallón E., Varona L. (2016). Efectos de cruzamiento y parámetros genéticos dela prolificidad en tres variedades de cerdo ibérico. Reunión Nacional de Mejora Genetica, June 2-3, 2016, Valencia (Spain).

Pérez-Enciso M., Misztal I. (2011). Qxpak5: Old mixed model solutions for new genomics problems. BMC Bioinformatics 12: 202.

Ponsuksili S., Reyer H., Trakooljul N., Murani E., Wimmers K., (2016). Single- and Bayesian multi-marker genome-wide association for haematological parameters in pigs. PLoS ONE 11: e0159212.

Puppe B., Langbein J., Bauer J., Hoy S. (2008). A comparative view on social hierarchy formation at different stages of pig production using sociometric measures. Livestock Science 113: 155-162.

Ragab M., Piles M., Quintanilla R., Sánchez J.P. (2018). Indirect genetic effect model using feeding behaviour traits to define the degree of interaction between mates: an implementation in pigs growth rate. Animal 13: 231-239.

Reiner G., Fischer R., Hepp S., Berge T., Köhler F., Willems H. (2008). Quantitative trait loci for white blood cell numbers in swine. Animal Genetics 39: 163–168.

Reiner G., Fischer R., Hepp S., Berge T., Köhler F., Willems H. (2007). Quantitative trait loci for red blood cell traits in swine. Anim. Genet. 38: 447–452.

Young J.M., Cai W., Dekkers J.C.M. (2011). Effect of selection for residual feed intake on feeding behavior and daily feed intake patterns in Yorkshire swine. Journal of Animal Science 89: 639-647.

5.3 Related publications and communications

Ballester M., Velasco-Galilea M., Piles M., Rafel O., González-Rodríguez O., Sanchez J.P. (2018). Genome-wide association study for growth and feed intake in a rabbit line selected for post-weaning daily gain. In: Book of Abstracts of the 69th Annual Meeting of the European Federation of Animal Science, p. 356. August 27-31, 2018. Dubrovnik, Croatia. Wageningen Academic Publishers.

Beauclercq S., Lefèvre A., Nadal-Desbarats L., Germain K., Praud C., Emond P., Le Bihan-Duval E., Mignon-Grasteau S. (2019). Does lipidomic serum analysis support the assessment of digestive efficiency in chickens? Poultry Science 98 : 1425-1431.

Fàbrega E., Pallisera J., Moles X., Escribano D., Cerón J., J.P. Sanchez (2017). New physiological indicators for welfare assessment: salivary chromogranin-A (cCGA) 11th International Veterinary Behaviour Meeting. September 12-16, 2017. Samorin, Slovakia.

Garreau H., Ruesche J., Gilbert H., Balmisse E., Benitez F., Richard F., David I., Drouilhet L., Zemb O. (2018). Microrabbits: a factorial design to evaluate genetic and maternal effects on growth and feed efficiency in a line selected for residual feed intake. In: Proceedings of the World Congress on Genetics Applied to Livestock Production, Volume Electronic Poster Session - Biology - Feed Intake and Efficiency 2, p. 222. February 11-16, 2018. Auckland, New Zealand. Massey University.

Garreau H., Ruesche J., Gilbert H., Balmisse E., Benitez F., Richard F., David I., Drouilhet L., Zemb O. (2019). Estimating direct genetic and maternal effects affecting rabbit growth and feed efficiency with a factorial design. Journal of Animal Breeding and Genetics. In press.

Gilbert H., Terenina E., Louveau I., Ruesche J., Gress L., Billon Y., Mormède P., Larzul C. (2017). Responses of pigs divergently selected for cortisol response or feed efficiency to an ACTH challenge. In: Book of Abstracts of the 68th Annual Meeting of the European Federation of Animal Science, p. 240. August 28 - September 1, 2017. Tallinn, Estonia. Wageningen Academic Publishers.

Gilbert H., Terenina E., Ruesche J., Gress L., Billon Y., Mormède P., Larzul C. (2018). Responses of pigs divergently selected for cortisol level or feed efficiency to a challenge diet during growth. In 11. World Congress on Genetics Applied to Livestock Production (WCGALP), Auckland, Nouvelle Zelande, 11-16 Février 2018 Volume Electronic Poster Session - Species - Porcine 2 - Poster, p.219.

Hela M., Ragab M. Sanchez J.P., (2018). Genetic parameters of feed intake patterns of Duroc sows during gestation and lactation. In: Proceedings of the World Congress on Genetics Applied to Livestock Production, Volume Electronic Poster Session - Species - Porcine 1, p. 155, February 11-16, 2018. Auckland, New Zealand. Massey University.

Herrera W., Ragab M., Sánchez J.P. (2019). Indirect genetic effects on the genetic correlations between production and feeding behavior traits in growing Duroc pigs. Animal (Submitted).

Herrera W., Sánchez J.P. (2018). Predicción de la Respuesta Correlacionada en Índice de Conversión usando Modelos de Interacción Social: Evaluación por Simulación. XIX Reunión Nacional de Mejora Genética Animal - León, Spain.

Herrera W., Ragab M., Sánchez J.P. (2017). Social genetic effects on productive and feeding behavior traits in growing Duroc pigs. In: Book of Abstracts of the 68th Annual Meeting of the European Federation of Animal Science, p. 240. August 28 - September 1, 2017. Tallinn, Estonia. Wageningen Academic Publishers.

Herrera W., Ragab M., Sánchez J.P. (2017). Efectos genéticos sociales en caracteres productivos y de comportamiento alimentario en cerdos Duroc en crecimiento. XVII Jornadas Sobre Producción Animal (AIDA), Zaragoza, Spain.

Mignon-Grasteau S., Beauclercq S., Urvoix S., Le Bihan-Duval E. (2019). Interest of serum color as an indirect criterion of selection of digestive efficiency in chickens. Poultry Science (submitted).

Ragab M., Piles M, Sanchez J.P. (2016). Alternative consideration of social genetic effects models in Duroc pigs. In: Book of Abstracts of the 67th Annual Meeting of the European Federation of Animal Science, p. 123. August 29 - September 2, 2016. Belfast, United Kingdom. Wageningen Academic Publishers.

Ragab M., Piles M., Quintanilla R., Sánchez J.P. (2018). Indirect genetic effect model using feeding behaviour traits to define the degree of interaction between mates: an implementation in pigs growth rate. Animal 13: 231-239.

Sanchez J.P., Legarra A., Piles M. (2018). Multiple trait single step Bayesian GWAS on pooled data. In: Proceedings of the World Congress on Genetics Applied to Livestock Production, Volume Genetic improvement programmes: Selection using molecular information, p. 489. February 11-16, 2018. Auckland, New Zealand. Massey University.

Master Theses:

William Andrés Herrera (2016). Estudio del determinismo genético de los caracteres de comportamiento en comedero de cerdos Duroc criados en grupo con sistemas de control individualizado de ingesta. Animal Science Department, Polichenic University of Valencia.

Júlia Martí Alcázar (2018). Análisis genético de la eficiencia alimentaria de la cerda en lactación y sus componentes. Animal Science Department, Polichenic University of Valencia. .