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Deliverable D1.1

Comprehensive phenotype recording for ELP traits in pig and chicken

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| Workpackage concerned | WP1 |
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Abstract

Half page/one page maximum

The main objective of WP1 is to unravel biological mechanisms underpinning Efficient Livestock Production (ELP) traits in pig and laying hen commercial populations, by conducting in depth combined analyses on phenotypes, genotypes, epigenotypes and transcriptomes.

One of the first steps of this WP1 was to produce a comprehensive phenotype recording in these two species, according to three animal experiments related to quality (egg, sperm and meat), egg productive longevity, feed efficiency, health and growth.

1) For the experiment related to boar semen quality, TNRC allocated 400 boars from a sire line and 100 boars from a dam line at 9 and 15 months of age for collection and phenotyping of a total of 1,000 samples. A total of 11 semen traits were assessed using CASA (Computer-Assisted Sperm Analysis), a standardized high-throughput phenotyping method used in artificial insemination stations for routine boar semen evaluation.

2) For layer experiment related to productive longevity, standard chicken phenotypes were recorded at 70 wk of age (700 animals) and 90 wk of age (500 animals, 200 being sacrificed for tissue sampling at 70 wk of age). All animals were individually phenotyped for feed intake, egg quality (albumen, yolk and shell strength), egg production, body weight and lipid storage. At 90 wk of age, bone strength and bone calcium rate & mineral rate were recorded by a specialized company (ZOOTEST, Ploufragan, France) for 350 birds. The fine yolk fatty acid composition was recorded by a specialized platform (METATOUL, Toulouse, France) on 300 eggs from 100 hens, and 1200 eggs (from 400 hens), including the previous 300 eggs, were analysed by near infrared spectroscopy (NIRS) for the development of an affordable prediction method of yolk fatty acid composition (see WP3)

3) For pig experiment related to growth, feed efficiency and meat quality, phenotype data related to growth and body composition as muscle percentage were extracted from SEGES/Danbred's databases from 109,905 animals. Additionally, data on feed efficiency and feeding behaviour were recorded in 757 boars.

Task 1.1 Comprehensive genome-wide sampling of phenotypic and genomic data of semen in pigs (M1-M40)

Task leader: TNRC / Involved Partners: WU, LiU

In the context of T1.1, TNRC allocated 400 boars from a sire line and 100 boars from a dam line at 9 and at 15 months of age for collection and phenotyping of a total of 1,000 samples. At present (August 2023), all 400 sire line boars have been collected and phenotyped at 9 months of age. Among them, 326* boars have also been collected and phenotyped at 15 months of age. Similarly, within the dam line, 78* boars have also been collected and phenotyped at 9 months of age, with 32* of those boars collected and phenotyped at 15 months of age.

Semen traits were assessed using CASA (Computer-Assisted Sperm Analysis), a standardized highthroughput phenotyping method used in artificial insemination stations for routine boar semen evaluation. CASA Systems have been used to monitor the progression of individual sperm cells and to estimate the percentage of sperm cells with total motility (total motility) and the percentage of sperm cells with progressive motility (progressive motility). In addition to this task, this system can also be used to assess the morphology of individual sperm cells and estimate the percentage of sperm cells with abnormalities (Total Abnormality Rate), but also head or tail abnormalities and distal or proximal cytoplasmic droplets. Finally, the total number of spermatozoa can be estimated and used to calculate concentration.

In summary, the dataset includes observations on a total of 11 different semen traits such as total number of sperm cells, concentration, volume, total motility, progressive motility, total abnormality, head abnormality, tail abnormality, distal droplets, proximal droplets and total droplets. Additionally, in the context of T1.4, TNRC provided supplementary phenotype records from 461,790 ejaculates obtained from 5,758 sire line boars and 72,004 ejaculates from 1,461 dam line boars. These extensive records were used to estimate genetic parameters and identify quantitative trait *loci* (QTL) associated with the observed semen traits.

*: Deviation mentioned and justified in the first report. Sampling of sperm from boars was started at an age of 9 months instead of 12 months as original planned. This to reduce the number of boars that could not be sampled at the 2nd round of sampling due to high replacement rates. The age differences between the two sampling time were kept at half a year as described in the original proposal.



Task 1.2 - Comprehensive genome-wide sampling of phenotypic and genomic data for productive longevity in laying hens (M1-M36)

Task leader: IA-AO / Involved Partners: INRAE, NOVO, LiU

Standard chicken phenotypes were recorded at 70 wk of age (700 animals) and 90 wk of age (500 animals, 200 being sacrificed for tissue sampling at 70 wk of age). All animals were individually phenotyped for feed intake, body weight, egg production and egg quality (albumen, yolk and shell strength). Briefly, egg production was recorded daily from 70 weeks to 90 weeks of age and laying rate (LR) was calculated weekly. Individual feed intake (FI) was measured during three periods of three weeks each, starting at 70, 80, and 90 weeks of age. The ingested quantity was estimated every 3-4 days by weighing the feeder at the start and end of the feeding period for each animal. Body weight (BW) was measured for every hen at the start and end of each feeding period, the egg weight (EW) was recorded daily. Thanks to these body and egg weights, the feed conversion ratio (FCR) and residual feed intake (RFI) were calculated for each of the 3 feed recording periods, at 70, 80 and 90 weeks of age.

Egg quality traits were measured by the company ZOOTEST on at least three eggs per hen collected at the beginning and end of each measurement period. Egg shell fracture force (ESFF) and egg shell static stiffness (ESSS) were measured by compressing eggs between two flat plates moving at constant speed, using a compression machine; ESFF is the maximum force recorded before eggshell fracture. ESSS presents deformation of the shell under a constant force of 15 Newton. Eggshell color (ESC) was obtained by recording redness, yellowness and lightness with a portable chromameter. Yolk weight, yolk percentage and Haugh unit (indicator of albumen height) were recorded.

Back fat reserve was recorded by ultrasound on alive hens at 70, 80 and 90 weeks of age. Abdominal fat reserve was recorded by weighting the abdominal adipose tissue after animal dissection at 70 and 90 weeks of age for 250 and 350 animals respectively.

Tibia bone strength was measured at 90 weeks of age on 350* slaughtered animals through two indicators (fracture Force and stiffness similarly to egg shell) by a specialized company (ZOOTEST). Were also recorded tibia percentage of dry matter, mineral matter and calcium.

Finally, the fine yolk fatty acid composition was recorded at 90 weeks of age by a specialized platform (METATOUL) on 300 PBS-diluted egg yolks from 100 hens by using an accurate but expensive biochemical analysis based on gas chromatography–flame ionization detection (GC–FID). Such an analysis provided fatty acid profiling in addition to the total fatty acids. In addition, the egg yolks from 400 hens at 90 wk of age (including the same 300 eggs measured by GC-FID) were also sampled without dilution in PBS and analysed by near infrared spectroscopy (NIRS) for the development of an affordable prediction method of yolk fatty acid composition (see WP3). Note that 1200 egg yolks were collected in PBS at 70 and 90 weeks of age for egg yolk triglycerides and cholesterol measurement using a colorimetric kit.

All these measures are available for WP1.4 analyses.

*: Deviation mentioned and justified in the first report. 350 bone samples were measured instead of 400 announced in the proposal, for a total of $15000 \in$ allocated finances, because of the price increase ($37 \in ->$ 44 \in). No consequence is expected on the final results, as the number of analysed birds is still large, representing 70% of the studied birds at 90 weeks of age.

Task 1.3 Comprehensive genome-wide sampling of phenotypic and genomic data for growth and feed efficiency in pigs (M10-M33)

Task leader: UCPH / Involved Partners: LiU, WU

Phenotype data has been extracted from SEGES/Danbred's databases on sex, daily gain, weight at start and weight at end of experiment, back fat thickness, and body conformation as muscle percentage for 19,864 Duroc pigs, 48,817 Yorkshire pigs and 42,055 Landrace pigs (110,736 in total). After removing animals with missing data (including missing genotype data), 109,918 pigs remained in the dataset with 19,749 Duroc, 48,427 Yorkshire and 41,742 Landrace.

Additionally, feed efficiency (relative growth rate, feed conversion ratio, residual feed intake and residual daily gain), and feeding behaviour (daily feed intake, daily eating time, daily feeding rate and daily number of eating episodes) were recorded for 757 pigs (262 Landrace, 262 Duroc and 233 Yorkshire).



Conclusion

All the phenotypes related to Efficient Livestock Production traits in pig and laying hen experiments of the WP1 have been recorded as expected, with some deviations for the WP1.1 and WP1.2 already mentioned and justified in the first report.