Norwegian University
of Life Sciences

**Master's Thesis 2019 30 ECTS**
Faculty of Biosciences

# Genetic variation in relation to stress from computed tomography in Norsvin Landrace pigs

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Animal breeding and genetics

# Acknowledgments

I would like to thank my supervisor Peer Berg and my co-supervisor Tale Marie Karlsson-Drangsholt for being absolutely fantastic supervisors. I appreciate you being so available and generous with your time. Thank you so much for all your input and having patience with me when I needed help, from study design to statistics to feedback on my writing, it is greatly appreciated.

I would also like to Eli Grindflek for suggesting Peer Berg and Tale Marie Karlsson-Drangsholt as my supervisor and co-supervisor, and for discussing potential thesis projects with me, and helping get a summer job at the testing station, which even though it was not in connection with the thesis, helped me understand my thesis.

I would like to thank my family, my girlfriend and my friends for helping me stay motivated and focused throughout this semester, and keeping me company when I needed a break.

Ås, June 2019

Andrew Heggli

# Abstract

Resilience towards stress is an important trait as it has implications for animal welfare, and can reduce labour costs. Though some traits included in breeding programs cover disease resilience, general resilience towards stress has yet to be included. As selective breeding has improved many other traits, it is feasible that it can be used to improve resilience towards stress. For a trait to be viable for selection it needs to have enough genetic variance and a high enough heritability to produce a response to selection. The aims of this thesis were therefore:

1. to develop novel phenotypes that can measure sensitivity to stress in pigs based on variation in feed intake (FI) data after computed tomography (CT-scanning) and potentially be used in selective breeding to increase stress resilience
2. to determine heritabilities, genetic variation and genetic correlations between the novel phenotypes and production traits such as growth.

Feed intake data from the Norsvin Delta testing station in Hamar, Norway was used to create novel phenotypes based on within boar regressions. There were two groups of novel phenotypes based on how they measured stress resilience; number of days in FI deficit post CT-scanning, and total accumulated deficit for the number of days in FI deficit post CT-scanning. Number of days in deficit measured the length of time boars were affected by stress from CT-scanning, and total deficit measured the amount boar FI was affected by stress from CT-scanning. Different methods of data treatment were applied to the two measures of resilience to find the method of measuring resilience that had the highest heritability and genetic variance, and would therefore be best suited to use in a breeding program. The best suited novel phenotypes were also used in multivariate analyses that included OCD score as a health trait, and standardised growth from 40 to 120 kg and standardised FI from 40 to 120 kg as production traits to ensure that they were separate traits from the novel phenotypes.

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# Introduction

Genetic variance is the soil upon which our planets diversity of life has been allowed to grow. We have been able to use this variance along with selective breeding to steer which direction the traits in our livestock change, and the more accurately we measure the traits the higher response we get to selection (Mrode, 2014). This is one of the reasons swine genetics company Norsvin uses computed tomography. It allows for accurate measurements, and the ability to measure traits that previously had to be registered after slaughter, such as osteochondrosis dissecans (OCD) and lean meat percentage. Selective breeding has previously been used to increase growth (Nguyen & McPhee, 2005; Rauw et al., 1998), disease resilience (Gharbi et al., 2015; Kolstad et al., 2005; Mallard et al., 1998; Stear et al., 2001), and even improve piglet survival through maternal skills (Fremmerlid, 2015; Ocepek & Andersen, 2018). It is therefore reasonable to consider that selective breeding can potentially be used to improve sensitivity to stress if there is enough genetic variation in the trait we select for, and the heritability is high enough. The alternative hypothesis was consequently that there is a genetic difference between boars in relation to their sensitivity to CT-scanning that can be used in selective breeding, and the null hypothesis was that there is no genetic difference between boars that we can be used in selective breeding. The aim of this study was therefore:

1. to develop novel phenotypes that can measure sensitivity to stress in pigs based on variation in feed intake (FI) data after computed tomography (CT-scanning) and potentially be used in selective breeding to increase stress resilience
2. to determine heritabilities and genetic correlations between the novel phenotypes and production traits such as growth.

Sensitivity to stress was based on within boar regressions, and measured in days in FI deficit, and accumulated FI deficit during those days. There is evidence that FI variation can be used as a measure of sensitivity as Putz et al. (2018) also based their novel phenotypes on variation in FI, though their phenotypes were in relation to disease rather than stress. They considered their novel phenotypes to be “black box” phenotypes that showed general resilience, rather than disease resilience as “many factors can cause variation in FI (…) including disease, heat stress, handling, and social interactions” (Putz et al., 2018). Likewise, the novel phenotypes presented in this thesis could be considered “black box” phenotypes that are not suitable as measures of sensitivity to a specific stressor, but rather show sensitivity to the combined stressors that are part of the CT-scanning process. Though stress can be defined in several ways (Martínez-Miró et al., 2016), this thesis considered the stress from CT-scanning to be acute (immediate stressors with quick onset and short duration) as our measurements of stress are examining effects on FI in the short term (days). CT-scanning is a multidimensional stressor that involves handling, potential stress from being in the same room as unfamiliar conspecifics,

separation distress, and a novel environment.

Handling is a part of the CT-scanning process in that the boars must be led from their treatment pens to the CT-scanning room. Boars must be sedated before scanning, so they were also handled when injected with the sedative Stresnil. These types of handling produces short term stress (Grandin, 2018). The variation in how individuals cope with this stress is affected by both environmental effects such as previous experiences, and genetic effects such as temperament (Grandin, 1997; Grandin & Shivley, 2015; Terlouw et al., 2008). The novelty of a new stimulus, in this case being in a new environment, though potentially less stressful than other stressors (Schrader & Ladewig, 1999) is still somewhat stressful to pigs. The severity novelty has as a stressor varies depending on the individual (Grandin, 1997; Grandin & Shivley, 2015; Terlouw et al., 2008).

In addition to being in a novel environment, boars that are scheduled for scanning are housed individually in the CT-scanning room

1. with either none or few of their pen mates and
2. unfamiliar conspecifics.

It is important to understand that these are two separate stressors. The first stressor is being separated from familiar conspecifics. There is strong evidence that this alone (without mixing unfamiliar individuals) is stressful to pigs (de Jong et al., 1998; Ruis et al., 1997; Ruis et al., 2001a; Ruis et al., 2001b; Schrader & Ladewig, 1999) and the stress from this separation can be referred to as separation distress (Grandin & Shivley, 2015). The second stressor is mixing unfamiliar individuals. There is strong evidence that mixing is stressful to pigs (Coutellier et al., 2007; Erhard et al., 1997; Giersing & Andersson, 1998; Jensen, 1994; McGlone, 1985; Stookey & Gonyou, 1994; Von Borell, 1995). The stress from mixing unfamiliar individuals affects reproduction (Von Borell, 1995), health (de Groot et al., 2001), behaviour (Giersing & Andersson, 1998), physiological traits (Coutellier et al., 2007) and production traits (Stookey & Gonyou, 1994). Mixing unfamiliar pigs results in fighting (Jensen, 1994; McGlone, 1985), which is a cause of stress, but the boars in the present study were housed in individual pens before scanning, and fighting was therefore not possible. Fighting is not the only cause of stress though; the mere presence of unfamiliar pigs may also be stressful (Erhard et al., 1997; Stookey & Gonyou, 1994), which could contribute to the stress of boars about to be CT-scanned.

In addition to the validity of the hypothesis being supported by FI variation being used as a measure of sensitivity previously and selective breeding previously improving various traits other than stress, selective breeding has successfully shown improvement in one of the stressors from CT-scanning. Mills and Faure (1991) successfully bred separate lines of Japanese quail for low and high separation distress (which they referred to as social reinstatement). This was measured by a treadmill test in which an isolated chick on a treadmill would try to get to its

conspecifics. Combined distance run and time spent on the treadmill were used as the measure of separation distress for the chick in test.

Current traits in breeding programs cover disease resilience, but not general resilience towards a variety of stressors (Berghof et al., 2018) and finding traits that can measure resilience and be implemented in breeding programs is therefore important. Animals in commercial production regularly encounter stressors, and breeding animals that are resilient towards those stressors would improve welfare (Grandin, 1997; Grandin, 2018). In addition, breeding for better stress resilience also has economic value as it can reduce labour costs (Berghof et al., 2018).

# Materials and Method

## Data collection

Data was collected from 4020 boars (start weight approx. 40 kgs, end weight approx. 120 kgs) from 1st September 2015 to 1st September 2018 at the Norsvin Delta station in Hamar, Norway. All boars in the data set completed the testing period. Any boars that did not complete testing due to illness or other issues were excluded. Feed intake (FI) and weight were measured using Osborne's FIRE® (Feed Intake Recording Equipment) System, which identified boars by ear tag produced by Caisley Eartag Ltd. FI was measured as the difference in feed weight in the station before and after feeding. A Norsvin quality control system excludes measurements of FI or weight if FI is not accurate due to the FI recording equipment needing recalibration or if the weight of the animal diverges from its growth curve e.g. due to weight loss from illness.

## Data treatment

All statistical treatments and analyses to produce new phenotypes were coded specifically for this thesis and executed in RStudio. FI per boar per day was plotted against age (in days). FI per boar per day was also plotted against weight per day (in kg). As the plots displayed unequal distributions for FI, a coefficient of variance (CoV = $\sigma/\mu$) of FI was calculated for three time periods based on age measured in days; CoV age 80-110 days, CoV age 111-140 days, and CoV age 141-180 days. These coefficients were approximately equal; and so, two different treatment methods were applied to the raw data. **Figure 1** shows the first method with natural logarithm ($\log_e$, from here on noted simply as log) transformed FI data (**Figure 1A**), the second method did not transform the data, but reduced the dataset by only including data from age $\geq$100 days (**Figure 1B**), and weight $\geq$60 kg. Due to the boars being moved from their testing pen at varying times of the day, the final day of FI data was inconsistent and thus excluded for all boars.

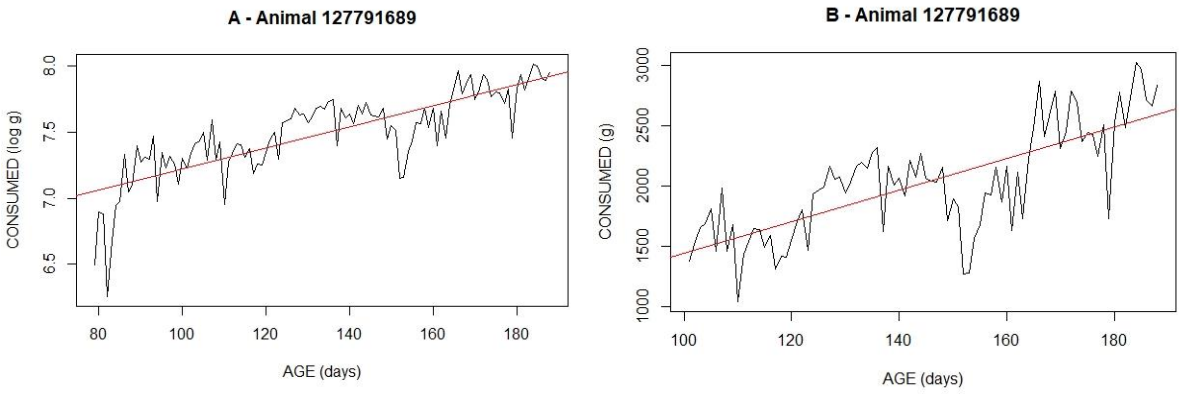


**Figure 1:** Feed intake vs age for animal 127791689 with **(A)** log transformed FI and **(B)** no transformation, but reduced number of days. The regression line represents linear regression predicted FI per day.

Based on the new datasets, four linear regressions were calculated for each individual boar:

1. Log transformed feed intake vs age in days
2. Log transformed feed intake vs weight
3. Non-transformed feed intake vs age (≥100 days)
4. Non-transformed feed intake vs weight (≥60 kg)

These regressions were each used to make a regression model. Using these models to make predicted values (**Figure 1**), each day boars either ate as predicted, were in FI surplus or in FI deficit. This is shown by whether FI is on, over or below the regression line in **Figure 1**. After scanning, most boars displayed an FI deficit, but some boars had no FI data on the first day after scanning, and others displayed a very minor FI surplus. As such, the data from the first day of feed intake was considered unreliable and therefore data on the first day post scanning were excluded for all boars. Boars with only three days of post scan FI data were left with only one day of post scan FI data after data treatment, due to the exclusion of data from the first day post scanning and the final day of FI data collection. These boars were therefore excluded from further analyses.

As shown in **Figure 2** the range of days in deficit varied between boars. Most animals had reached predicted FI by day five for all linear regressions (**Figure 2**), therefore boars that had less than five days of post scan data and did not reach predicted FI within those days would not have the opportunity to show their phenotype. Boars this applied to were consequently excluded. Some boars were moved directly from the scanning room to waiting rooms for export or transference to a different testing station, and therefore no FI data was available from these boars. FI data after scanning is therefore only from boars that were moved back to their original pens after scanning.

These boars were later moved to other rooms depending on whether they were going to export or selected for breeding. When they were moved varied, and thus there was variation in how many days of data boars had after scanning. Due to this and the fact that some animals never reached predicted FI, a comparison between boars at the furthest ends of range would be unbalanced, and therefore only data from days 2-7 post scanning were included when calculating the new phenotypes. After data treatment the remaining data set included boars that:

- had < five days of data but reached predicted FI
- had ≥ five days of data and reached predicted FI
- had ≥ five days of data but did not reach predicted FI

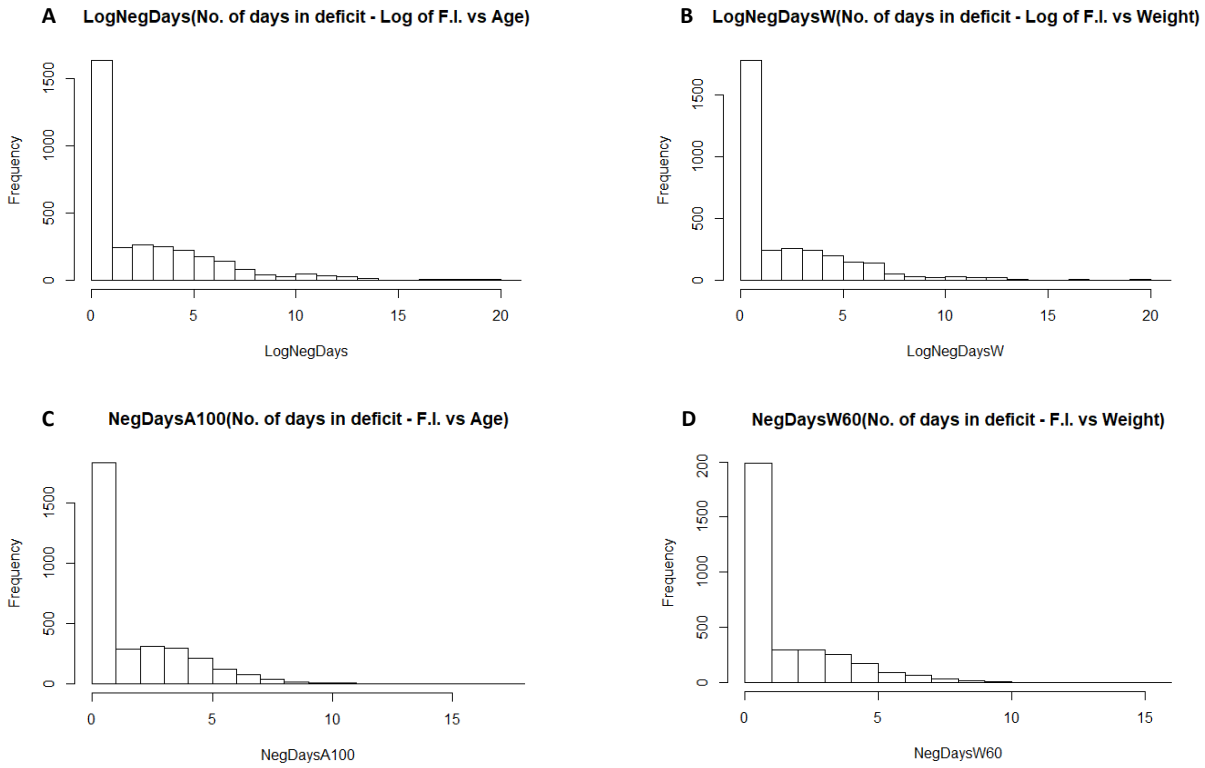


**Figure 2:** Frequency of days in deficit based on each linear regression before reducing the range of days in deficit, but post removal of the first and last day of post scan FI data. Two regressions for log transformed data, two for reduced data. Zero days in deficit are for boars that ate as predicted the first day post scanning.

## New phenotypes - post data treatment

Two groups of novel phenotypes were created (**Table 1**); number of days in FI deficit until the boar reached predicted FI and accumulated total deficit - until the boar reached predicted FI. Days in FI deficit measured the length a boar was in deficit, accumulated total deficit measured the amount (in grams) the boar was in deficit during the days until it reached predicted FI. Any days in deficit after the boar reached predicted FI were not included for either phenotype group. This meant that boars that ate as predicted or were in FI surplus on the first day post scanning had zero days in deficit, and their FI deficit was therefore also set to zero.

Each boar had eight novel phenotypes in total; two phenotype groups:

1. number of days in FI deficit – from day two post scanning until predicted FI was reached, or until day seven post scanning if predicted FI was not reached
2. accumulated FI (g) deficit post scanning – amount of total deficit from day two post scanning until predicted FI was reached, or until day seven if predicted FI was not reached

with four phenotypes per group based on:

1. log transformed FI vs age
2. log transformed FI vs weight
3. non-transformed reduced age range FI vs age
4. non-transformed reduced weight range FI vs weight

**Table 1:** Novel phenotypes, how they were measured and which regressions they were based on

| *Phenotype group* | **Phenotype** | **Description** |
|---|---|---|
| *Days in deficit* | Log days (age) | Measured in number of days in FI deficit, based on log transformed FI vs age regressions |
| | Log days (weight) | Measured in number of days in FI deficit, based on log transformed FI vs weight regressions |
| | Reduced days (age) | Measured in number of days in FI deficit, based on non-transformed (≥100 days) FI vs age regressions |
| | Reduced days (weight) | Measured in number of days in FI deficit, based on non-transformed (≥60 days) FI vs weight regressions |
| *Accumulated total deficit* | Log deficit (age) | Measured in log of grams in FI deficit, based on log transformed FI vs age regressions |
| | Log deficit (weight) | Measured in log of grams in FI deficit, based on log transformed FI vs weight regressions |
| | Reduced deficit (age) | Measured in grams in FI deficit, based on non-transformed (≥100 days) FI vs age regressions |
| | Reduced deficit (weight) | Measured in grams in FI deficit, based on non-transformed (≥60 days) FI vs weight regressions |

The remaining post treatment data set consisted of 2845 boars with one data point per phenotype.

## Statistical analyses

All statistical analyses were performed using DMUv6 R5.2 from Center for Quantitative Genetics and Genomics (QGG), Department of Molecular Biology and Genetics, Aarhus University. The files loaded into DMU consisted of:

- a pedigree file for the estimation of the A matrix, consisting of four columns:
    1. The animal ID of all animals in the pedigree file, with the oldest animal first and the youngest last
    2. The sires of the corresponding animal in the ID column
    3. The dams of the corresponding animal in the ID column
    4. The birthdate of the corresponding animal in the ID column
- a data file consisting of all the new phenotypes, observations of traits, and an animal ID connecting the phenotypes and traits to the corresponding animal
- a directive file that determined which file the traits and phenotypes were stored in, which file the pedigree information was stored in, and which phenotypes, traits and models were to be used for each analysis. Every analysis had its own directive, but the pedigree and data file were the same for every analysis

Two modules within DMU were used for every analysis; DMU1 and DMUAI. DMU1 is a module that must always be used prior to using any of the other modules. DMU1 read and saved the information contained in the directive file, produced summary statistics for the trait(s) being analysed, and stored the number of levels for every factor in the model. DMUAI used average information restricted maximum likelihood (AI-REML) to estimate (co)variance components.

All statistical results (e.g. variance components) were based on mixed model equations (MME). Univariate, bivariate and multivariate analyses were performed, starting with univariate analyses for each novel phenotype. Several models were tested for the univariate analyses, always with the same fixed effects, but varied random effects. The first analyses tested inclusion of pen, litter, then both litter and pen as random effects, but in all tests pen and litter led to convergence problems and both effects had very little variance (<0.01). They were therefore excluded from the final model. The final model was the same for every univariate analysis for each novel phenotype:

$$X = H_i(f) + B_j(f) + C_k(f) + \beta_{NL}(fr) + \beta_{NL2}(fr) + ID_l(r) + e_{ijkl}(r)$$

Where;

(f) = fixed effect

(fr) = fixed regression
(r) = random effect

X = Novel phenotype
$H_i$ = Herd and year the animal was born in (i=1,…113)
$B_j$ = Month the animal was born (j=1,…12)
$C_k$ = Compartment animal was in during testing period (k=1,…5)
$\beta_{NL}$ = Regression coefficient for number liveborn in the litter
$\beta_{NL2}$ = Regression coefficient for number liveborn in the litter squared
$ID_l$ = Identification of the animal (l=1,…13715)
$e_{ijkl}$ = Error (ijkl=1,…2845)

Univariate analyses produced genetic variances and error variances, and heritabilities were calculated based on these outputs. Standard error of the heritability was not included in DMU output and therefore the "Approximate Variance for Heritability Estimates" equation from Klei (2019) was used to calculate heritability variance, the variance was then used to calculate standard error in Excel 2016 for Windows 10.

After univariate analyses, bivariate analyses were performed. The model used for each phenotype was the same as in the univariate analyses. There were three groups of bivariate analyses and four analyses within each group (**Table 2**)

**Table 2:** Groups of bivariate analyses and analyses within each group

| *Bivariate analysis group* | **Bivariate analysis** |
|---|---|
| *Age vs Weight* | Log days (age) vs Log days (weight) |
| | Log deficit (age) vs Log deficit (weight) |
| | Reduced days (age) vs Reduced days (weight) |
| | Reduced deficit (age) vs Reduced deficit (weight) |
| *Log vs Reduced* | Log days (age) vs Reduced days (age) |
| | Log days (weight) vs Reduced days (weight) |
| | Log deficit (age) vs Reduced deficit (Age) |
| | Log deficit (weight) vs Reduced deficit (weight) |
| *Days vs Deficit* | Log days (age) vs Log deficit (age) |
| | Log days (weight) vs Log deficit (weight) |
| | Reduced days (age) vs Reduced deficit (age) |
| | Reduced days (weight) vs Reduced deficit (weight) |

Two multivariate analyses were performed:

1. Log days (age) vs OCD score vs FI40120 vs D40120
2. Log deficit (age) vs OCD score vs FI40120 vs D40120

Both analyses tested four traits; a novel phenotype, an osteochondrosis dissecans (OCD) trait and two production traits. The two production traits were total FI (kg) from 40-120kg (FI40120) and standardised number of days from 40-120kg, a measure of growth (D40120). The OCD trait used was an OCD score that measured presence and severity of OCD based on CT scans.

For multivariate analyses one novel phenotype measured in days, and one in deficit were selected to be able to compare and notice differences between the length based (days) and amount based (deficit) methods of measuring stress after scanning. Which novel phenotypes to include was determined based on genetic variance and heritability, which were derived from the univariate analyses. Log days vs age was selected because it had the highest genetic variance out of all novel phenotypes measured in days (**Table 4**). Log deficit vs age was selected as the deficit phenotype because it had the highest genetic variance and heritability out all novel phenotypes measured in deficit (**Table 4**). The novel phenotypes and production traits were modelled with the univariate model, but the production traits had increased levels for $H_i$ and $C_k$:

For novel phenotypes;
$H_i$ = Herd and year the animal was born in (i=1,…113)
$C_k$ = Compartment animal was in during testing period (k=1,…5)
For production traits;
$H_i$ = Herd and year the animal was born in (i=1,…115)
$C_k$ = Compartment animal was in during testing period (k=1,…6)

The OCD score used a different model:

$$\text{OCD score} = HYS\_OCD_i(f) + OCD\_TDS_j(f) + \beta_{AGE\_OCD}(fr) + \beta_{AGE\_OCD2}(fr) + \beta_{NL}(fr) + \beta_{NL2}(fr) + \beta_{WT\_150}(fr) + \beta_{WT\_1502}(fr) + ID_k(r) + e_{ijk}(r)$$

Where;

(f) = fixed effect
(fr) = fixed regression
(r) = random effect

OCD score = Osteochondrosis dissecans score (range = 0,…19)
$HYS\_OCD_i$ = Season and year during CT-scanning (herd was the Delta station and therefore the same for all animals) (i=1,…17)
$OCD\_TDS_j$ = Operator that calculated the OCD score, season and year of CT-scanning (j=1,…21)
$\beta_{AGE\_OCD}$ = Regression coefficient for age at OCD scoring
$\beta_{AGE\_OCD2}$ = Regression coefficient for age at OCD scoring squared
$\beta_{NL}$ = Regression coefficient for number liveborn in the litter
$\beta_{NL2}$ = Regression coefficient for number liveborn in the litter squared

$\beta_{WT\_150}$ = Regression coefficient for standardised weight at 150 days of age
$\beta_{WT\_1502}$ = Regression coefficient for standardised weight at 150 days of age squared
$ID_k$ = Identification of the animal (k=1,…13715)
$e_{ijk}$ = Error (ijk=1,…3956)

# Results

**Table 3** shows that there was little difference between age based and weight based means of novel phenotypes. Though the difference was small, novel phenotypes based on age consistently had larger mean days in deficit and consequently larger mean total deficits than corresponding phenotypes based on weight. This was also true for standard deviations based on age vs corresponding standard deviations based on weight. All novel phenotypes had large standard deviations. The range of day phenotypes were the same due to data treatment only including post scanning days from 2 to 7.

Reduced mean days based on age and reduced mean days based on weight are both calculated based on FI data from the same boar, so the difference in mean is due to two differences:

1. Difference between the datasets; age based reduced data was from ≥100 days age, and ≥60 kg for weight. Most boars reached 60 kg around the 100$^{th}$ day, but not exactly on the 100$^{th}$ day, and therefore their dataset based on age, and their data set based on weight were similar, but not identical.
2. Age continuously increasing, but weight fluctuating.

Mean log days were consistently higher than mean reduced days.

OCD and production traits had more observations than novel traits. This is due to 1111 animals having data for production and OCD traits but lacking the required data to produce novel phenotypes e.g. not having the FI or weight data required. The range and standard deviation of the production traits were small compared to their means., and OCD score had a low mean compared to its range, but a moderately large standard deviation.

**Table 3:** Summary statistics for novel, OCD and FI phenotypes.

| *Phenotype groups* | **Phenotype** | **No. of phenotypes** | **Mean** | **SD** | **Min** | **Max** |
|---|---|---|---|---|---|---|
| *Days in deficit* | Log days (age) | 2845 | 2.283 | 2.321 | 0 | 6 |
| | Log days (weight) | 2845 | 2.022 | 2.238 | 0 | 6 |
| | Reduced days (age) | 2845 | 1.826 | 2.033 | 0 | 6 |
| | Reduced days (weight) | 2845 | 1.587 | 1.92 | 0 | 6 |
| *Accumulated total deficit* | Log deficit (age) | 2845 | -0.402 | 0.522 | -6.2 | 0 |
| | Log deficit (weight) | 2845 | -0.355 | 0.507 | -6.18 | 0 |
| | Reduced deficit (age) | 2845 | -800.585 | 1071.457 | -6100.213 | 0 |
| | Reduced deficit (weight) | 2845 | -684.845 | 1002.985 | -6124.832 | 0 |
| | OCD score | 3956 | 3.288 | 2.81 | 0 | 19 |
| | FI40120 | 3956 | 166.91 | 11.336 | 138.39 | 233.47 |
| | D40120 | 3956 | 71.987 | 6.301 | 50.73 | 102.79 |

**Table 4** shows estimated heritabilities, genetic variances, error variances, genetic correlations and error correlations from bivariate analyses between novel phenotypes. Heritabilities range from 0.074 to 0.105, genetic correlations from -0.998 to 1, and error correlations from -0.857 to 0.931. Genetic variances are measured in different units and therefore range is not applicable, but all genetic variances have moderate to large standard errors comparative to their respective means. The same applies for error variances and their standard errors. All heritabilities are low and have low standard errors. Low standard errors indicate that the estimated heritabilities are likely an accurate estimate of the true heritability.

All genetic and error correlations had low standard errors, indicating that the correlations are accurate. Genetic and error correlations between phenotypes based on the same measure (day vs day or deficit vs deficit) were all positive, and correlations between day and deficit phenotypes were all negative, which is to be expected; days are measured in positive numbers, deficit in negative numbers, so as days increase, the deficit decreases, giving a negative correlation. Genetic correlation was strongest between log days (age) and log days (weight), a correlation of 1 indicating that these phenotypes are the same trait. The error correlation for the same traits was not 1 but was still positive and very high, meaning environmental effects affect these traits in the same direction.

The genetic correlation between log deficit (age) and log deficit (weight) could not be calculated due to the model not converging, because the variance parameters of both traits converged towards zero. This means the two phenotypes could not be distinguished from one another.

**Table 4:** Estimated heritability (SE), genetic variance (SE) and error variance (SE). Estimated genetic correlations (SE) between novel phenotypes (upper diagonal). Error correlations (SE) between phenotypes (lower diagonal). "NA" = Bivariate analysis terminated due to lack of convergence. "-" = No analysis performed. † = Age vs weight. ‡ = Log transformed vs reduced. § = Days vs deficit.

| *Phenotype* | $h^2$ | $\sigma^2_g$ | $\sigma^2_e$ | **Log days (age)** | **Log days (weight)** | **Reduced days (age)** | **Reduced days (weight)** | **Log deficit (age)** | **Log deficit (weight)** | **Reduced deficit (age)** | **Reduced deficit (weight)** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Log days (age)* | 0.099 (0.014) | 0.523 (0.176) | 4.783 (0.195) | 1 | 1.000 (0.025)† | 0.971 (0.050)‡ | - | -0.979 (0.036)§ | - | - | - |
| *Log days (weight)* | 0.105 (0.014) | 0.52 (0.169) | 4.446 (0.184) | 0.864 (0.007)† | 1 | - | 0.966 (0.048)‡ | - | -0.965 (0.041)§ | - | - |
| *Reduced days (age)* | 0.096 (0.013) | 0.399 (0.135) | 3.743 (0.151) | 0.726 (0.013)‡ | - | 1 | 0.975 (0.026)† | - | - | -0.998 (0.021)§ | - |
| *Reduced days (weight)* | 0.091 (0.012) | 0.337 (0.117) | 3.351 (0.133) | - | 0.712 (0.013)‡ | 0.712 (0.013)† | 1 | - | - | - | -0.968 (0.032)§ |
| *Log deficit (age)* | 0.094 (0.007) | 0.025 (0.008) | 0.24 (0.009) | -0.801 (0.009)§ | - | - | - | 1 | NA† | 0.974 (0.044)‡ | - |
| *Log deficit (weight)* | 0.081 (0.006) | 0.021 (0.007) | 0.231 (0.009) | - | -0.777 (0.010)§ | - | - | NA† | 1 | - | 0.960 (0.050)‡ |
| *Reduced deficit (age)* | 0.080 (5.412) | 90552.185 (33032.3) | 1046504.439 (39636.6) | - | - | -0.857 (0.007)§ | - | 0.773 (0.010)‡ | - | 1 | 0.989 (0.017)† |
| *Reduced deficit (weight)* | 0.074 (4.903) | 73880.218 (27978.9) | 919367.724 (34307.7) | - | - | - | -0.848 (0.007)§ | - | 0.781 (0.010)‡ | 0.931 (0.003)† | 1 |

**Table 5** shows estimated genetic and error correlations between log days (age), OCD score, FI40120 and D40120. **Table 6** shows the estimated genetic and error correlations between log deficit (age), OCD score, FI40120 and D40120. The genetic correlation between log days (age) and OCD score was close to zero, indicating that these are two separate traits. The corresponding standard error was large so there is potential that the correlation is further from zero than estimated. There was a weak positive genetic correlation between log deficit (age) and OCD score, but the corresponding standard error was large, so it is uncertain whether the true correlation is closer to zero or an even stronger positive correlation. Genetic correlations between the novel phenotypes and both FI40120 and D40120 were moderate to low, indicating that days in deficit after scanning could potentially be a separate trait from FI and growth. This is however uncertain as these correlations had large standard errors and are less reliable due to the difference in sample size between the novel phenotype and the other traits (**Table 3**). Genetic correlations between OCD score and both FI40120 and D40120 were low for both novel phenotypes but had large standard errors. These correlations had balanced sample sizes and therefore the large standard error is potentially due to the sample size being too small. Genetic correlations between FI40120 and D40120 for both novel phenotypes were positive and

moderately strong, and had a lower standard error than all other genetic correlation standard errors. Error correlations between FI40120 and D40120 for both novel phenotypes were positive, strong and identical. Both had a low standard error. All other error correlations were close to zero and had small standard errors.

**Table 5:** Estimated genetic correlations (SE) in white background and estimated error correlations (SE) in grey background between Log days (age), OCD and FI phenotypes.

| *Phenotype* | **Log days (age)** | **OCD score** | **FI40120** | **D40120** |
|---|---|---|---|---|
| *Log days (age)* | 1 | -0.093 (0.172) | 0.216 (0.160) | 0.387 (0.165) |
| *OCD score* | 0.042 (0.034) | 1 | -0.019 (0.106) | 0.229 (0.113) |
| *FI40120* | 0.004 (0.037) | -0.047 (0.04) | 1 | 0.334 (0.091) |
| *D40120* | -0.098 (0.034) | -0.065 (0.038) | 0.738 (0.023) | 1 |

**Table 6:** Estimated genetic correlations (SE) in white background and estimated error correlations (SE) in grey background between Log deficit (age), OCD and FI phenotypes.

| *Phenotype* | **Log deficit (age)** | **OCD score** | **FI40120** | **D40120** |
|---|---|---|---|---|
| *Log deficit (age)* | 1 | 0.260 (0.168) | -0.199 (0.160) | -0.261 (0.168) |
| *OCD score* | -0.081 (0.034) | 1 | -0.026 (0.106) | 0.231 (0.113) |
| *FI40120* | -0.016 (0.036) | -0.045 (0.040) | 1 | 0.332 (0.092) |
| *D40120* | 0.067 (0.033) | -0.066 (0.038) | 0.738 (0.023) | 1 |

# Discussion

Novel phenotypes were created from FI data to measure genetic differences between boars in resilience towards stress from the CT-scanning process. Future research might vary in e.g. which sedative is used, or how the boars are housed before scanning, however, the general methods used to create these phenotypes can be replicated. In addition, the methods in the current study are not limited to CT-scanning as it is not the only process that is stressful to pigs. The same general method can be applied to resilience to other stressful events as long as FI data per animal per day is available.

To reach the aims of this study, it was necessary to develop phenotypes that could detect genetic differences between boars' resilience towards stress associated with the CT-scanning process. When the genetic differences are large, it is easier to differentiate between boars, and therefore larger genetic differences were preferable. Larger genetic differences indicate larger genetic variance, which is also preferable because this yields a stronger response to selection and often a higher heritability (Mrode, 2014). Several phenotypes were therefore created using different methods so the method(s) that maximise genetic differences could be discovered.

## Means, standard deviations and range

The means, standard deviations and range of the novel phenotypes were calculated to get an indication of whether the novel phenotypes showed a difference between boars (**Table 3**). Before reducing the range of number of days in deficit (**Figure 2**), it was clear that there was a difference between boars in regard to the length of time they were in deficit after scanning. Boars had a varying number of days of data collection after scanning, and it was uncertain how much this contributed to variation in number of days in deficit. Data treatment was therefore applied to mitigate the variation between boars in how many days of data they had post scanning and only include day two post scanning to day seven. Data treatment included removing data from the first data post scanning, which is supported by the fact that the sedative used before CT-scanning has previously been shown to increase FI the day after it has been administered (Gonyou et al., 1988) and therefore reduced the affect stress had on boars the day after scanning. Post data treatment it was clear that there was still a difference between boars in regard to number of days in deficit. Both number of days in deficit and total deficit for all novel phenotypes had large standard deviations in relation to their corresponding means (**Table 3**). The standard deviations of all novel phenotypes were larger than the absolute mean value of their corresponding mean, which indicated a larger percentage of the observations being zero or close to zero for both number of days in deficit and total deficit phenotypes. This stands to reason as there was a higher frequency of boars with zero days in deficit (**Figure 2**), and consequently these boars also had zero in total deficit. A higher frequency of boars having zero days in deficit is favourable as this shows that many boars have

resilience towards the stress from CT-scanning. This did not include all boars, as the range of the novel phenotypes for number of days in deficit (**Table 3**) showed that there were boars with six days in deficit, and these boars potentially had even more days in deficit as all boars with six days in deficit did not reach expected FI. It could be argued that the FI surplus from the first day after scanning for boars with zero days in deficit could have been included, but this would effectively alter their phenotype from number of days in deficit to number of days in deficit plus one day of surplus. This would then also have to apply to boars that did not have zero number of days in deficit. Including their surplus the day after their last day in deficit would have lowered the amount of total deficit these boars were in, and thus the statistical power. Addition of a day of surplus would also add an aspect of compensatory FI for boars that experienced a deficit and we would then not just be measuring the extent to which a boar is affected by a stressful event, but also their ability to recover from it.

### Heritabilities and variances

The standard deviations and range of the novel phenotypes (**Table 3**) indicate that there was a difference between boars, but genetic variance and heritabilities were necessary to determine whether there were genetic differences between boars, and whether that genetic difference could be used in selective breeding. Genetic variances and heritabilities were low, but present (**Table 4**), the latter ranging from 0.074 to 0.105. Both genetic and error variances for novel phenotypes had moderately large standard errors (**Table 4**). This indicates that the heritabilities were somewhat inaccurate because they were calculated using the genetic and error variances, but the equation does not take into account the standard errors of the variances. Since the heritabilities were inaccurate, they could be found to be higher or lower in future research. The preferable outcome would be for the genetic correlation to increase, and the error to decrease, and the standard errors to be smaller than estimated in this thesis; then the heritability would simultaneously increase and be more reliable. If this were to occur, it is unlikely that the heritabilities would display a large increase, as even the phenotype with the smallest difference between genetic and error variance (Log days (weight)) displayed an error variance almost ten times larger than the genetic variance. This would indicate that the environment has a greater impact on the boars' response to CT-scanning than genetics, though the effect of genetics is still present. The fact that there is a genetic effect, and a genetic difference between boars in regard to resilience to CT-scanning means we cannot reject the alternative hypothesis. Although the low heritability would yield a slow response to selection, there would still be some response, and it would therefore be possible to include a novel phenotype for resilience among breeding goals in a breeding program.

### Difference between novel phenotypes

The genetic differences and heritabilities showed that the novel phenotypes have the potential to be used in breeding programs, but adding a couple or a single phenotype would be

preferable to adding several as the more traits you select for in a breeding program the less weight you can give each trait. It was therefore preferable if the novel phenotypes were highly correlated, but this would also make it more difficult to choose which phenotype was best as there would be little difference between them.

This was the case as all genetic correlations between novel phenotypes were >|0.9| and had small standard errors (**Table 4**). Though the correlations, like the heritabilities, were somewhat affected by the large standard errors of the variances, they were still more reliable than the heritabilities because they are the quotients of the covariance between phenotypes and their standard deviations. This means that unlike heritabilities, they give us information about the relationship between phenotypes rather than information about a single phenotype. The strong genetic correlations infer that genes that affect one trait also affect correlated traits, and therefore a change in one trait would result in a change in the other trait, either in the same direction if the correlation is positive, or in the opposite direction if the correlation is negative. As error correlations were also strong, environmental effects that affect one trait would also affect correlated traits in the either the same or the opposite direction depending on the correlations. Due to the similarity between phenotypes, it was not clear which phenotype would be the best candidate to use in breeding programs.

Two aspects of the novel phenotypes could then be used to select a candidate; Heritability and genetic variances, and understanding what differentiated the novel phenotypes. The novel phenotypes could be differentiated into three groups (**Table 2**); whether they were measured in number of days in deficit or accumulated total deficit, whether they were based on log transformed or reduced data, and whether they were based on FI vs age or FI vs weight regressions. Looking at the general trends of each group in regard to genetic variance and heritability could potentially give us an idea of which phenotype to choose.

Phenotypes based on age consistently had higher genetic variances than corresponding phenotypes based on weight (**Table 4**), and three out of four age phenotypes had higher heritabilities than their corresponding heritability based on weight (**Table 4**). It would therefore stand to reason that the phenotype to include in a breeding program should be based on age rather than weight. Phenotypes measured in days and based on log transformed data (**Table 1**) had higher genetic variances (**Table 4**) than corresponding phenotypes based on non-transformed, reduced data. Comparing genetic variances between Log deficit and Reduced deficit (**Table 4**) phenotypes would be difficult as they are on different scales, but heritabilities were consistently higher for phenotypes based on log transformed data than phenotypes based on reduced data (**Table 4**). The cause is that on a day where there is a FI surplus that's close to zero for non-transformed data, there will be a deficit for FI based on transformed data. The consequence of this is that a boar this applies to will have a higher number of days in deficit for its log transformed vs non-transformed data, and consequently a higher total deficit. It therefore stands to reason that log based phenotypes have higher genetic variances than their

non-transformed counterparts, and thus, a higher heritability. Therefore log based phenotypes are preferable over reduced based phenotypes.

Day and deficit phenotypes measured two different aspects of resilience to stress; number of days in deficit measured the length of time boars were affected by stress from CT-scanning, and total deficit measured the amount boar FI was affected by stress from CT-scanning. Therefore one phenotype per measure were selected for multivariate analysis with OCD and production traits (**Table 5 & 6**). Age and log based phenotypes generally had higher heritabilities and genetic variances than corresponding phenotypes, and thus log days (age) and log deficit (age) were selected. Multivariate analyses were necessary because if the novel phenotypes were highly correlated to production traits, then they would not be a true measure of stress resilience and including them in breeding goals would be redundant. Though log days (age) displayed higher heritability than log deficit (age) (**Table 4**), if log deficit (age) had a weaker correlation to production traits than log days (age), it would be preferable to use in a breeding program as it would be a better measure of stress resilience.

### Multivariate analyses

OCD scores had a low mean and standard deviation (**Table 3**), which are favourable as this indicates most boars do not suffer severely from OCD (Olstad et al., 2014). OCD score was a trait included in the multivariate analyses because it is one of the traits derived from the CT scans, and is a health trait. Correlations between stress resilience phenotypes and a health phenotype were of interest because strong correlations would indicate that OCD and resilience to stress are affected by the same genes. This turned out not to be the case. There was a very weak negative genetic correlation between OCD scores and number of days in deficit, and a weak positive genetic correlation between OCD scores and total deficit (**Table 5 & 6**). This indicates that to a small degree boars genetically predisposed to higher OCD scores are also genetically predisposed to have smaller total deficits, and to a lesser degree fewer number of days in deficit. This is unfavourable as it makes breeding towards lower OCD scores and higher stress resilience more difficult.

Both novel phenotypes used in the multivariate analyses had moderately low genetic correlations to the production traits (**Table 5 & 6**). This indicates that to a small degree the same genes that affect stress resilience affect the production traits. Considering that the novel phenotypes were based on FI, this was expected. It would be preferable if they were completely separate traits, but a small genetic correlation indicates they are at least somewhat separate, and therefore that the novel phenotypes could be used as a measure of stress resilience. In addition, unlike the correlations to OCD scores, these correlations were favourable; the quicker a boar grows and the less FI they have from 40 to 120 kg, the fewer number of days in deficit, and the smaller the deficit post scanning. This makes breeding towards stress resilience easier as it would be in the same direction as the production traits.

Genetic correlations between OCD score and D40120 were moderately low, but positive for both multivariate analyses (**Table 5 & 6**). This indicates that boars that have lower OCD scores have quicker growth, which should also indicate a smaller deficit post scanning, yet based on the correlation between OCD score and log deficit (age) (**Table 6**), it should be boars with higher OCD scores that have quicker growth. These results are therefore conflicting, and as standard errors for the correlations are moderately large, future studies could display correlations that are higher or lower than estimated in this thesis and thereby illuminate whether estimates closer to the true correlations produce less conflicting results.

### Future research

As the phenotypes created here are novel, there was sparse literature to compare genetic variances and heritabilities, but Putz et al. (2018) also created novel phenotypes based on FI that measured resilience. Genetic variances were not presented in their study, but their novel phenotypes had slightly higher heritabilities (0.15 to 0.26) than those estimated here. Several differences between their study and this thesis could have led to the differences in heritabilities. Their main cause of deviation from predicted FI was illness, whereas our main cause was stress from scanning. Their phenotypes used FI both above and below predicted FI over a longer period of time and included several reactions to illness. Ours only included FI below predicted FI and only one stress event. Their methods of measuring resilience were also different, they used root mean square error (RMSE) and quantile regression (QR), for both feeding duration as well as FI. Though there were many differences, there was a common goal of improving resilience through creating novel phenotyping that have potential for selective breeding. In this regard both studies could be replicated using the same study design, but swap novel phenotypes, and thereby discover which phenotypes yield higher heritabilities and genetic variances, and are thus better suited to be included in breeding schemes.

Putz et al. (2018) noted that using variation in FI as a measure of resilience results in stressors having a greater impact on FI in older animals, as variance in FI increases with age. Thus resilience measures that include data from both a young and old age, such as their novel phenotype $RMSE_{FI}$, puts an increasingly greater weight on stressors the older the animal is. Our phenotypes only included data from older animals and thus our phenotypes did not suffer the same problem. Older animals have previous life experience, and can therefore may be desensitised and/or habituated towards stressors to a greater degree than younger animals (Grandin & Shivley, 2015). Due to younger animals potentially being less habituated to stressors, future studies on stress resilience based on data from Norsvins Delta station could potentially create novel phenotypes from FI data after boars arrive at Delta. Many of the same stressors during CT-scanning, such as handling, a novel environment and being housed close to unfamiliar conspecifics are present when boars first arrive. As the boars are younger, they could potentially show greater variation in their resilience to these stressors than resilience to stress

from CT-scanning when they are older. This would be preferable as it would be easier to differentiate between boars.

## Conclusion

Log day (age) and log deficit (age) displayed low enough correlations with production traits and OCD that they can be considered separate from production traits and OCD. Their heritabilities and genetic variances were high enough that they can be used as quantitative measures of resilience to stress from the CT-scanning process. As this process includes a combination of stressors, these novel phenotypes should be considered quantitative measures of resilience towards the overall stress, rather than towards the specific stressors, included in the CT-scanning process.

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