Physiological response to the weaning in two pig lines divergently selected for residual feed intake

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Divergent selection for RFI and weaning

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vi. Abstract and Keywords

Breeding efficient pigs is a way to reduce dietary costs and environmental waste. However, optimisation of feed efficiency must not be linked to a decrease of the ability of animals to cope with stress, such as the weaning. This study characterizes the response after weaning of pigs from two lines divergently selected for residual feed intake (RFI) during growth. Animals of the low (L) RFI line are more efficient than animals from the high (H) RFI line. Thirty six piglets from each line, weaned at 28 days of age were individually housed and fed a conventional dietary sequence. Their performance, behaviour, health and oxidative status, immune and nutritional parameters were followed during three weeks. Daily feed intake and growth rate of pigs from the LRFI line were 35% and 40% lower compared with HRFI ($P<0.001$). Pigs from the LRFI-line had lower total tract apparent digestibility (–6% for OM)

and suffered more from undernutrition with a 167 and 55% higher plasmatic concentration of NEFA and urea compared with HRFI (P<0.01). In the first week after the weaning, they had more diarrhoea, and had a higher inflammatory status with concentration of haptoglobin 52% higher (P< 0.001). These piglets then seemed to adapt to the weaning conditions and to recover during the second and third weeks. Both lines had similar zootechnical performance and physiological characteristics at the end of the post-weaning period. To conclude, the physiological responses to the weaning differed between lines. Pigs from the LRFI line, selected for greater feed efficiency were more sensitive to the weaning stress. They were also more resilient as they finally adapted to the new condition and recovered to show similar performance results as pigs of the HRFI line.

Feed efficiency, pig, residual feed intake, selection, weaning

vii. Main text

Introduction

In commercial pig farms, the weaning is a critical period for pig due to an important sensibility to digestive disorders. Then the weaning period has been often associated with the use of antibiotics to prevent or to cure diarrhoea. In the worldwide context of reduction of the use of antibiotics to limit bacterial resistance and preserve public health, there is an increased interest to select piglets that have important ability to cope with the stress of weaning (maternal separation, litter mixing, new, transportation, diet change, etc.).The immediate consequences of the weaning stress are decreased voluntary feed intake, growth check and increased sensitivity to enteric pathogens that compromise animal health and welfare (Molist et al., 2014). These reflect physiological perturbations as altered gastrointestinal tract with villus atrophy, compromised barrier function, reduced digestive and absorptive capacity, microbiota dysbiosis (Pluske, Hampson, & Williams, 1997; Lallès, Bosi, Smidt, & Stokes, 2007; Lallès & Guillou, 2015), systemic inflammation, and oxidative stress (Zhu, Zhao, Chen, & Xu, 2012; Buchet et al., 2017). This acute phase is usually followed by a recovery or adaptive phase corresponding to an adaptation of the pig to its new rearing, feeding and social conditions (Burrin & Stoll, 2003; Montagne et al., 2007). When focusing on the kinetic of the piglet response after weaning, robustness at weaning could result 1) from a greater resistance of piglets to the stressing factors, corresponding to a lower sensitivity during the acute phase and/or 2) from a greater resilience to these factors, corresponding to a more rapid recovery during the adaptive phase (Sauvant & Martin, 2010).

Improving feed efficiency is a major goal in pig production because of its relationships to reduced feed costs and environmental impacts. Residual feed intake (RFI), defined as the

difference between observed feed intake and that predicted on the basis of requirements for maintenance and growth, is a criteria to divergently select pigs for feed efficiency (Gilbert et al., 2007). For a similar growth rate, pigs selected for low RFI (LRFI; more efficient) eat less and spend less time eating (Young, Cai, & Dekkers, 2011) than pigs selected for high RFI (HRFI; less efficient). In a previous study we showed that pigs from the more efficient line were more immediately affected by weaning stress (growth check and more diarrhoea), but managed to recover afterwards (Gilbert et al., 2019a). The present study was designed to investigate the line differences in terms of behaviour, health and oxidative status, immune and nutritional parameters of the pigs to understand the underlying mechanisms driving the differences of robustness at weaning.

Materials and methods

The experiment was performed in INRAE experimental facilities in Saint-Gilles (France) in compliance with the French directive on animal experimentation and care (2013-118) after evaluation by the ethic committee in animal experimentation (C2EA – 07), and received an authorization from the French Ministry of Research (project number 2016010512258334).

Animals, housing, diets and experimental design

Two Large White pig lines have been divergently selected for RFI (LRFI, pigs eating less than predicted for their production level, and HRFI, pigs eating more than predicted) during 8 generations (Gilbert et al., 2007; Gilbert et al., 2017). Thirty six piglets from each line (total of 72 pigs, equal number of females and castrated males), all born during the same week, were weaned at 28 days of age. The piglets were selected on the following criteria: maximisation of the number of litters (9 sows for LRFI and 7 sows for HRFI), balanced number of castrated males and females per litter, and average BW the day before the weaning (day-1) per line representative of the average weaning BW in their contemporary line. Piglets did not have access to creep feeding during lactation.

From the weaning day (day 0) until the end of the experiment (day 33), piglets were housed in individual pens (80 × 60 cm, slatted floor) to measure individual feed intakes. Each pen was equipped with a trough and a water nipple. Individual pens were separated by transparent partitions (Plexiglass®) preventing physical contact with other pigs so limiting the stress of individual housing but permitting visual contacts. The room temperature was maintained at 28°C during the first week post-weaning and was then progressively decreased to 24°C (1°C/day).

Pigs were fed a starter diet immediately after weaning, and a two days transition starting at day 11 was applied between the starter and the weaning diets. Diets were provided by CCPA (Janzé, France). Diets were based on wheat, barley, soybean meals and extruded soybean

seeds (see Control diets compositions in Gilbert et al., 2019a). They contained highly digestive feedstuffs (cooked cereals, biscuits meals, whey, and potato proteins) to enhance appetite and digestibility. The starter and weaning diets contained respectively 19.0 and 16.6 % of crude protein, 1.22 and 1.05 % of digestible lysine, and 10.44 and 9.70 MJ of net energy. Titanium dioxide (0.4%) was included as an inert marker for digestibility calculations in the two diets. The diets were offered as pellets. Pigs had free access to feed and water throughout the experiment.

Observations, measurements, and sample collection

Individual feed intake (feed offered minus refusals) was recorded daily during the first three weeks after weaning, and then weekly for the remainder of the experiment. Pigs were weighed on days 0, 6, 12, 19, 26, and 33, and average daily gain was calculated for each period. In addition, all piglets were individually weighed at birth for better understanding of the general growth response of the animals. The faeces consistency was recorded at days 1, 2, 6, 12, and 19, using a three levels grid (normal, soft or diarrheic faeces). Rectal temperature was measured on days 1, 5, 6 and 7 after weaning.

Behavioural observations of pigs were performed on 12 animals per line using 2-min instantaneous scan sampling for 30 minutes per day (equivalent to 15 observations per day for each pig), as previously described by Pastorelli et al. (2012). Behaviour of pigs was observed during 4 periods : days 0 to 2, days 4 to 8, days 11 to 13 (feed transition), and days 18 to 21, excluding days when blood was sampled (days 6 and 18, details below). The scan started 5 minutes after the feed distribution in the morning. Three postures (lying, sitting, and standing) and five behaviours (inactive, eating, drinking, social behaviour like attempting to sniff counterpart through the transparent side of the pen, and exploring) were recorded. The traits observed during the 30 min scan sampling were expressed as a percentage of time spent for each posture and activity. Then the percentage of time in each posture and activity was calculated for each period of observation as the average of the percentages of the days with observations.

Blood (5 mL into heparin and twice 5 mL into EDTA tubes) was collected at the jugular vein the day before weaning (day-1), and at days 6 and 19, between 0800 and 1000 hours, before feed distribution in the morning. Samples were kept on ice immediately after collection. Plasma from one tube on EDTA was instantaneously used for blood formula analyses (white and red cells counting). The two other tubes were centrifuged (2600 × g, 4°C, 10 min). Plasma from the second EDTA tube was stored at -20°C for assays of glucose, non-esterified fatty acid (NEFA), urea, insulin, IGF-1, immunoglobulins G and M, and haptoglobin. Plasma from the heparin tube was stored at -80°C until analyses of the biological antioxidant potential (BAP) and peroxide concentration (Reactive Oxygen Metabolites: dROM).

Approximately 10 g of faeces were collected at days 6 and 19 for volatile fatty acids (VFA) and ammonia concentration assays. A phosphoric acid solution (0.5%, 1mL for 1g of faeces) was immediately added to stop fermentation and samples were stored at -20°C. After faecal collection for VFA and ammonia analyses on days 6 and 19, a plastic bag (stoma bag Hollister, Paris, France; ref. 3778) was fixed on the animal anus using adhesive strip (Tensoplast®, BSN Medical, Le Mans, France) to collect faeces on days 6 and 7, and 19 and 20 for digestibility analyses. The bag was removed after defecation and faeces were stored at -20°C. After lyophilisation, samples of two consecutive days were pooled, grinded and stored at +4°C.

Biological analyses

The total number of erythrocytes (red blood cells), leucocytes (white blood cells), and neutrophil granulocytes was measured with a haematology automated cell counter calibrated for pigs (MS9®; Melet Schloesing Laboratories, Osny, France). Kits were used to assay plasma concentration of glucose (Glucose RTU; Biomérieux), NEFA (NEFA-HR(2), Wako Chemicals GmbH), urea (UREA 981818, Thermo Fisher Scientific), and insulin (Insulin-CT, IBA Molecular). All these compounds were analysed with Konélab 20XT automate (Thermo Fisher Scientific, Courtaboeuf, France). Plasma IGF-1 concentration was determined after an acid–ethanol extraction using the IRMA IGF-1 kit (Immunotech, Prague, Czech Republic). Plasma haptoglobin was assayed by colorimetry (Tridelta PHASE Haptoglobin, Tridelta Development). Immunoglobulins G (IgG) and M (IgM) were assayed by ELISA (Bethyl Elisa quantitation; Interchim, Montluçon, France). The dROMs and BAP were assayed using a test kit (Callegari, Parma, Italy; distributor Deltavit Laboratory, Janze, France) following the method described by Sauerwein, Schmitz, & Hiss (2007). The concentration of hydroperoxides generated by the peroxidation of lipids, proteins and nucleic acids (Alberti et al., 1999) was measured with dROM. Results of the test were expressed in CARRU (Carratelli Unit, 1 CARRU = 0.08 mg H_2O_2/100 mL of plasma). The BAP results from the combined effects of antioxidants such as uric acid, ascorbic acid, proteins, alpha-tocopherol and bilirubin (Benzie & Strain, 1996). Results are expressed in µmol.L of equivalent vitamin C used as an iron-reducing reference agent. An Oxidative Stress Index (OSI) representing the amount of oxidative products per anti-oxydant capacity was calculated as the ratio of dROM to BAP (CARRU /·µmol-1·L of Vit C-1; Sharma, Pasqualotto, Nelson, Thomas, & Agarwal, 1999).

For the determination of VFA, frozen samples of faeces were first added (1 g/1 mL) with 0.4% (wt/vol) crotonic acid. After 2 h at 4°C, the samples were mixed and then twice centrifuged at 16,500 × g for 10 min at 4°C. The VFA dissolved in the supernatant were then

185 determined by gas chromatography using 4-methylvaleric acid as internal standard (Jouany,
186 1982). Total concentration of VFA was expressed as a percentage of faecal DM.
187 Diets and faeces were analysed for DM (method 934.01), OM and minerals (method 942.05),
188 and crude proteins (N × 6.25; method 990.03) using the AOAC (2000) methods. The
189 concentration of Ti02 in diets and faeces was determined photometrically (Cobas Mira,
190 Horiba ABX, Montpellier, France) according to the method of Njaa (1961). The apparent total
191 tract digestibility of nutrients was calculated as previously described (Montagne et al., 2014).
192
193 Statistical analysis
194 All variables except faecal consistency scores were analysed with linear mixed models (lmer
195 function of lme4 package (Bates, Maechler, Ben Bolker, & Walker, 2015), lsmeans, car and
196 multcompView packages) of R software (R Core team 2015, version). Four pigs, two from
197 each line, were excluded from the experiment, three due to sanitary problem (acute
198 diarrhoea), and one due to persistent anorexia following weaning. The line, the time (period
199 or day), and the line by time interaction were used as fixed effects; the animal and the litter
200 were introduced as random factors. The body weight at weaning was used as a covariate for
201 performance traits. Pair-wise comparisons of least square means were performed using a
202 Tukey test. Chi2 tests were used to test, at each day of observation, the effect of the line on
203 the number of pigs having normal, soft or diarrheic faeces. Differences with P-values lower
204 than 0.05 were considered as significant, and differences with P-values between 0.05 and
205 0.10 were reported as trends.
206
207 **Results**
208
209 Growth performance
210 The LRFI piglets were heavier than the HRFI piglets at weaning (9.08 ± 0.75 vs 7.96 ± 1.09
211 kg, P<0,005, Table 1). A BW difference was already observed between lines at birth
212 (1.55±0.23 and 1.33±0.28 kg for LRFI and HRFI, respectively, P<0.01). During the 33 days of
213 post-weaning period, BW adjusted to BW at weaning did not significantly differ between lines
214 (P=0.19). The BW measured at days 12 and 19 were lower for LRFI pigs compared with
215 HRFI (P=0.001). Other BW did not differ between lines (P>0.10). Accordingly, a lower ADG
216 was observed for the LRFI line from days 0 to 11 (– 40% compared with HRFI line, P<0.001),
217 and lower average daily feed intake was observed in the same period (-34%, P<0.001). The
218 difference in daily feed intake was observed until day 7 after weaning (Figure 1). For other
219 performances, there was no line difference in performance after day 12 (Table 1).
220
221 Digestion, fermentation and nutritional status

At the end of the first week after weaning (days 6 and 7), total tract apparent digestibility coefficients of dietary components were significantly lower in pigs from the LRFI line compared with HRFI pigs, from -6% for OM to -13% for N ($P < 0.001$; Table 2). Total tract apparent digestibility coefficients increased with time and did not significantly differ between lines on days 19 and 20, with an average of 82.2, 85.2, 60.7 and 79.6 % for dry matter, organic matter, minerals, and N, respectively (see Table S1 for details).

The total concentration of VFA and ammonia did not differ between lines, and it increased between days 6 and 19 ($P < 0.001$; Table 2). The ratio between total VFA and ammonia tended to be lower for pigs of the LRFI line compared with HRFI pigs at the two times of measurement ($P = 0.06$). The proportions of the different VFA differed with time ($P < 0.001$) between the lines. In LRFI pigs, the proportion of acetate and branched chain fatty acids were lower at day 19 than day 6, and those of propionate and butyrate were greater, whereas in the HRFI pigs only the concentration of branched chain fatty acids significantly changed between days 6 and 19 (see Table S2 for more details).

The time effect on plasma glucose concentration differed between the two lines ($P=0.02$). At the day before weaning (day -1), LRFI pigs had greater plasma glucose concentration than HRFI pigs (Table 2). Plasma glucose concentration was lower on day 6 compared with day -1 for the two lines but did not differ between lines at days 6 and 19. The NEFA and urea concentrations did not differ between lines the day before weaning, nor at day 19. By contrast, greater concentrations were observed at day 6 in LRFI pigs (+167% for NEFA and + 55% for urea compared to HRFI, $P < 0.001$). The IGF-1 concentrations were similar between lines on days -1 and 19, but lower values were observed for the LRFI pigs compared with HRFI pigs on day 6 ($P=0.001$). Plasma insulin concentration was affected neither by the time nor by the line.

Fecal score, blood indicators of inflammation and oxidative status

Two episodes of diarrhoea were observed (Figure 2). During the first episode, from days 0 to 3 after weaning, the prevalence of pigs having diarrheic faeces was more important for the HRFI line (12/34 pigs on day 3) than for the LRFI line (1/34 pigs) ($P<0.001$). Conversely, during the second episode, from days 6 to 9, the prevalence of pigs with diarrhoea was higher for the LRFI than for HRFI pigs (11 vs 2/34 pigs at day 7). The measurement of rectal temperature did not evidence any fever episode (results not shown). Haptoglobin plasma concentration increased from weaning to day 6 and decreased from day 6 to day 19 (Table 3), with a different time effect between lines ($P<0.001$): concentrations were greater for the LRFI line at day 6 (+52% compared with HRFI).

The white blood cell counts differed with the two lines with a significant interaction between time ($P=0.007$). In HRFI pigs, white blood cell counts increased from day -1 to 6, and was

unchanged at day 19, whereas it did not differ between day -1 and 6 and increased between days 6 and 19 in the LRFI line. The red blood cell counts were overall greater in LRFI pigs (P=0.012) and increased over time for both lines (P= 0.023). The concentrations of IgG did not differ between lines just before weaning and on day 6, but lower concentrations were measured in the plasma of LRFI pigs on day 19 (-35% of the concentrations of HRFI pigs, P<0.019). Plasma concentration of IgM was lower the day before weaning in LRFI pigs compared with HRFI pigs. It increased between days 0 and 6, and the difference between lines was no more significant on days 6 and 19.

The concentrations of dROM changed similarly for the two lines ($P \geq 0.58$; Table 3) during the experiment period ($P \leq 0.002$). The concentration of dROM increased from day -1 to day 6, and then remained unchanged between days 6 and 19. The anti-oxydative ability (BAP) was similar between days -1 and 6 and tended to increase between day 6 and day 19. Consequently, the ISO ratio was maximal at day 6 (P<0.05) and did not differ between the lines.

Posture and activity traits

The time spent to postures and activities did not differ between lines the first week after weaning (see Figure S1 for data). Pigs of the LRFI line tended to spend less time standing from days 11-13 compared with HRFI (29% vs 42% of time, respectively; P = 0.09). Inversely, the third week after weaning (days 18-21), pigs of the LRFI line spent more time standing (48% vs 32% of the time for LRFI and HRFI respectively, P<0.05), whereas pigs of the HRFI line stayed mainly lying (65% of the observation time vs 45% for HRFI and LRFI, respectively, P<0.05, Figure S1a). The inactive time and the time spent on social behaviour and exploration did not differ between lines at any period. The third week after weaning (days 18-21), LRFI pigs spent more time eating (38%) compared with HRFI pigs (24%, P<0.01, Figure S1b).

Discussion

This study confirmed that the divergent selection for RFI during growth affected differently the response of pigs to weaning. Pigs from the more efficient line (LRFI) were more affected by weaning (growth check and more diarrhoea) during the first post-weaning week, and managed to recover afterwards, as reported before (Gilbert et al., 2019a).

During the first week after weaning, the larger changes in BW observed for LRFI compared with HRFI pigs suggested a greater sensitivity or a lower resistance to the weaning stress of pigs selected for a greater feed efficiency. These differences might be partially explained by a difference in voluntary feed intake. Actually, low and variable feed intake is a major cause

of post-weaning disorders and growth check (Pluske et al., 1997; Le Dividich & Sève, 2000). In the present study the anorexia was probably greater because of the absence of creep feeding during lactation. Both lines have low feed intake after weaning (less than 300 g/d per pig), but the LRFI pigs ingested particularly little compared to HRFI pigs, despite a similar time spent eating during the first week after the weaning. Note that behavioural observations need to be interpreted with care because they result from punctual observations (scan) and animals were housed in individual cases. In addition, this might have enhanced weaning stress and limited stimulation by counterparts. The LRFI pigs spent probably more time on feed investigation and rummage in the trough rather than feed ingestion. Differences in feeding behaviours have been reported between these lines during the growing phase, with HRFI pigs spending more time eating than LRFI pigs (Meunier-Salaün et al., 2011). Similar differences were described in another experiment divergent selection in Yorkshire pigs performed at the Iowa State University (Young et al., 2011).

Decrease in voluntary feed intake is a common animal response to a stressful event. We hypothesize that LRFI pigs were more affected by the stress induced by weaning. Difference in plasma cortisol in response to an ACTH injection was evidenced between HRFI and LRFI pigs aged of 6 weeks (ie two weeks after weaning), indicating genetically driven line differences in the adrenocortical axis reactivity. Pigs from both lines had similar basal cortisol concentrations but the responses to ACTH of the LRFI pigs was greater and persisted longer than that of HRFI pigs (Gilbert et al., 2019b) . Increased stimulation of the corticotropic axis is a primary endocrine stress response pathway that has also consequence on metabolism, inflammation and immunity (Mormède et al., 2011), and could also be partly responsible for the greater response of LRFI pigs during the acute post-weaning phase.

The increase in plasma haptoglobin concentration between weaning and day 6 after weaning was more marked in the LRFI pigs. Accordingly, as described by Dirkzwager, Veldman, & Bikker (2005), the post-weaning anorexia leads to an inflammation of the intestinal mucosa and a decrease of the digestive capacity that could be the causes of diarrhoea. As inflammation is known to generate an oxidative stress on farm animals (Lykkesfeldt & Svendsen, 2007), it could explain the increase of oxidative stress between days 0 and 6. Such oxidative stress the first week after weaning is in accordance with Zhu et al. (2012) and Buchet, Belloc, Leblanc-Maridor, & Merlot (2017). Even if the pro-oxidant molecules (dROM) did not differ between lines at day 6, the increase from days 0 to 6 was consistently numerically more marked for the LRFI pigs than the HRFI (+ 34% vs 25%).

These line differences can also be related to the greater incidence of diarrhoea in the LRFI pigs observed from days 5 to 9 after weaning. Surprisingly, diarrhoeas were not associated with an increase of white blood cells, contrasting with others studies (Le Huërou-Luron et al., 2004). By contrast the number of white blood cells increased from day 0 to 6 in HRFI pigs.

That might be the consequence of the diarrheic episode observed in this line day 3 after the the weaning. Diarrhoeas observed from days 5 to 9 after weaning in the LRFI pigs were not associated with fever and might be the consequence of changes in the digestive capacity and dysbiosis, rather than bacterial infection (Dirkzwager et al., 2005; Molist et al., 2014). The lower total tract digestibility of nutrients in LRFI pigs compared with HRFI reinforced this hypothesis of weak digestive capacities. The difference between the two lines was particularly pronounced for nitrogen, with a difference of 12.9 units, which is twice the difference measured for OM. Such a difference in total tract digestibility probably reflects a difference in the true digestibility and so on availability of AA for proteins synthesis affecting animal growth. Proteins that were not digested before the ileum were fermented in the distal part of the gastro-intestinal tract with ammonia and branched chain VFA as the major end products (Williams et al., 2001). The trend observed for greater concentration of branched fatty acids and lower ratio between VFA and ammonia in the faeces of LRFI pigs at day 6 is in accordance with the observed difference of nitrogen digestibility. A higher ratio between VFA and ammonia is considered as favourable for the digestive health (Williams et al., 2001). The lower ratio observed for the LRI line compared with HRFI could also be related to the greater incidence of diarrhoea. Differences in the concentrations of fermentation end-products in the faeces could be linked to differences in the microbiota composition observed after weaning. Contrary to LRFI piglets whose fecal microbiota remained unchanged between day 0 and day 6, the fecal microbiota in the HRFI line changed indicating probably a faster adaptation to the new post-weaning conditions (Kubasova et al., 2018). After day 6 no predominant genera relating to pathogenic strains were evidenced in these faeces. Because changes in genera were often associated with post-weaning diarrhoea (Karasova et al. 2021), this reinforces the hypotheses of a nutritional rather than an infectious origin of diarrhoea.

The lower feed intake and digestive capacity of LRFI might explain the nutritional status difference of the two lines evidenced at day 6, and in turn the lower growth of LRFI pigs until day 11. The greater plasma concentration of NEFA and urea measured in LRFI pigs may result from a greater mobilization from body lipids and proteins, and a greater amino acid catabolism for energy supply. Such differences between lines were also observed in Gilbert et al. (2019). As the metabolism is considered to contribute to around 75% to the growth check observed after weaning (Pastorelli, van Milgen, Lovatto, & Montagne, 2012), the greater growth check in LRFI pigs was probably also greatly associated with greater inflammation and diarrhoea, in association with changes in digestibility and in metabolic status.

As indicated by the growth response to weaning, LRFI pigs had a greater sensitivity to weaning but recovered afterwards and adapted to the post-weaning conditions. Three weeks after weaning, the phenotypic differences between lines were limited: the lines had similar growth performance, digestibility and VFA profiles, and were in a similar metabolic, oxidative and health status. The genetic improvements of efficiency have been suggested to have unfavourable consequences in the pig's ability to cope with stressors and to maintain their health (Rauw, Kanis, Noordhuizen-Stassen, & Grommers, 1998; Doeschl-Wilson et al., 2009; Prunier, Heinonen, & Quesnel, 2010). However, experimental challenges did not validate this hypothesis in divergent RFI lines (Gilbert et al., 2017, Dunkelberger et al., 2015). When exposed to various challenges, no advantage of one line or the other was previously identified (response to inflammation and heat stress: Merlot, Gilbert, & Le Floc'h, 2016 ; Renaudeau, Frances, Dubois, Gilbert, & Noblet, 2013; Campos et al., 2014 ; response to weaning: Gilbert et al., 2019a), or there was an advantage to the LRFI pigs (Chatelet et al., 2018 for a low hygiene challenge). In a similar and independent selection of divergent selection on RFI on Yorkshire pigs (Cai, Casey, & Dekkers, 2018), lines were tested for their ability to respond to an experimental infection by the PRRSV 1 to 3 weeks after weaning (Dunkelberger et al., 2015) and with *Mycoplasma hyopneumoniae* and *Lawsonia intracellularis* (Helm et al., 2018). The present study confirms that even if the short term response to the weaning (day 6) differed between lines, the overall capability of pigs to cope with the weaning disturbance, considered as a key component of robustness (Friggens et al., 2017; Revilla et al., 2019) is similar in the two lines. This reinforces the idea that it is possible to simultaneously improve productivity and robustness (Hermesch, Li, Doeschl-Wilson, & Gilbert, 2015).

Conclusion

This study aimed at identifying the biological mechanisms underlying the temporal difference of the response to weaning between lines. Selection on RFI has impacted the immediate response but has globally not affected the robustness of pigs to the weaning challenge. Pigs from the LRFI line, selected for greater feed efficiency during the growing period, were more sensitive to the weaning stress with an important anorexia, growth check, and higher frequency of diarrhoea at the end of the first week after weaning. There also seem to be more resilient as they finally adapt to the new condition and recover to show similar performance results as pigs of the HRFI line. Differences of the responses were related to some indicators of digestion, to the nutritional, oxidative, inflammatory statuses and to some fermentative indicators.

Animal Welfare Statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

viii. References

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570 **ix. Tables**

571

572 Table 1. Performances (least-square means, n=34) after weaning (day 0) of pigs from two
573 lines divergently selected for residual feed intake (RFI)

	Line†			P-value‡			
	High RFI	Low RFI	SEM	Line	Time	Line×Time	BW_0
BW (kg)							
Day 0 (weaning) §	8.77^{g}	8.32^{g}	0.24				
Day 6	9.41^{fg}	8.53^{g}	0.24				
Day 12	11.44^{e}	10.17^{f}	0.24	0.188	<0.001	0.119	<0.001
Day 19	13.97^{c}	12.80^{d}	0.23				
Day 26	17.28^{b}	16.15^{b}	0.24				
Day 33	21.53^{a}	20.63^{a}	0.24				
Average daily feed intake (g/d)							
Days 0 to 11	288^{b}	190^{c}	16	<0.001	<0.001	0.036	<0.001
Days 12 to 33	842^{a}	895^{a}	16				
Average daily gain (g/d)							
Days 0 to 11	239^{b}	139^{c}	13	<0.001	<0.001	<0.001	<0.001
Days 12 to 33	498^{a}	483^{a}	13				
Gain to Feed ratio							
Days 0 to 11	0.84^{a}	0.73^{b}	0.003	0.005	<0.001	0.007	0.419
Days 12 to 33	0.56^{c}	0.57^{c}	0.003				

574 † Two pig lines differing for their residual feed intake (RFI): less efficient High RFI *vs* more
575 efficient Low RFI

576 ‡ Probability values for the effect of genetic line, time (days for BW or periods from 0 to 11
577 and 12 to 33 days for other criteria), interaction between line and time, and BW at weaning
578 (BW_0), introduced as a covariate in the statistical model

579 §Average BW at weaning was 7.96±1.09 kg for High RFI pigs and 9.08±0.75 kg for Low RFI
580 pigs

581 ^{a,g}Values within a trait with different superscripts differed significantly at P<0.05

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583

584 Table 2. Apparent total tract digestibility coefficients, fermentation parameters and nutritional
585 status (least-square means, n=34) after weaning (day 0) of pigs from two lines divergently
586 selected for residual feed intake (RFI) †

	Line†			P-value‡		
	High RFI	Low RFI	SEM	Line	Time	Line×Time
Total tract digestibility of organic matter, %						
Day 6	81.3[b]	75.0[c]	0.83	<0.001	<0.001	<0.001
Day 19	85.4[a]	84.9[a]	0.76			
Total tract digestibility of nitrogen, %						
Day 6	72.1[b]	59.2[c]	1.80	<0.001	<0.001	<0.001
Day 19	80.3[a]	78.9[a]	1.72			
Total volatile fatty acids, μmol/g faeces						
Day 6	69.23[b]	63.20[b]	8.24	0.606	<0.001	0.858
Day 19	178.28[a]	169.43[a]	7.56			
Relative proportion of acetate/propionate/butyrate/ branched chain fatty acid, %§						
Day 6	61/20/9/7	63/18/9/8				
Day 19	60/22/11/5	59/22/11/5				
Ammonia, μmol/g faeces						
Day 6	18.84[b]	24.22[b]	3.49	0.276	<0.001	0.296
Day 19	46.64[a]	45.02[a]	3.20			
Glucose (mg/l)						
Day -1	1436.5[b]	1654.4[a]	36.8			
Day 6	1234.7[c]	1275.7[c]	36.9	<0.001	<0.001	0.021
Day 19	1242.3[c]	1301.2[bc]	37.7			
Non esterified fatty acid (μmol/l)						
Day -1	351.0[a]	340.7[a]	23.6			
Day 6	129.6[b]	346.9[a]	23.8	0.758	<0.001	<0.001
Day 19	38.5[b]	39.2[b]	24.5			
Urea (mg/l)						
Day -1	151.1[a]	171.0[a]	9.0			
Day 6	135.7[b]	210.7[a]	9.0	0.118	<0.001	0.002
Day 19	76.2[c]	96.8[c]	9.2			
Insulin (μU/l)						
Day -1	6.13	7.83	0.94			
Day 6	6.73	8.12	0.94	0.201	0.868	0.983
Day 19	6.68	8.26	0.97			

Insulin-like growth factor-1 (IGF-1) (ng/ml)						
Day -1	152.2[b]	161.3[b]	7.9			
Day 6	107.7[c]	57.3[d]	8.0	0.418	<0.001	0.001
Day 19	200.6[a]	177.4[ab]	8.3			

† Two pig lines differing for their residual feed intake (less efficient High RFI *vs* more efficient Low RFI)

‡ Probability values for the effect of genetic lines, time (days of faeces or blood collection) and interaction between line and time

§ See supporting information Table S2 for more details

[a,d]Values within a criterion with different superscripts differed significantly at P<0.05

Table 3. Main indicators of health and oxidative status (least-square means, n=34) after weaning (day 0) of pigs from two lines divergently selected for residual feed intake (RFI) †

	Line†			P-value‡		
	High RFI	Low RFI	SEM	Line	Time	Line×Time
Haptoglobin (mg/ml)						
Day -1	0.36^{c}	0.24^{c}	0.14			
Day 6	1.53^{b}	2.33^{a}	0.14	0.561	<0.001	<0.001
Day 19	1.47^{b}	1.11^{b}	0.14			
White blood cell counts (10^3/mm^3)						
Day -1	14.47^{d}	15.57^{bcd}	0.95			
Day 6	17.81^{abc}	14.28^{cd}	0.95	0.414	0.001	0.007
Day 19	18.35^{ab}	19.01^{a}	0.97			
Red blood cell counts (10^3/mm^3)						
Day -1	6.91^{b}	7.23^{ab}	0.09			
Day 6	7.13^{b}	7.52^{a}	0.09	0.012	0.023	0.837
Day 19	7.18^{ab}	7.50^{a}	0.09			
Immunoglobulins G (g/l)						
Day -1	5.65^{ab}	4.58^{bcd}	0.32			
Day 6	5.47^{abc}	4.17^{cd}	0.32	0.019	0.151	0.062
Day 19	6.12^{a}	3.98^{d}	0.35			
Immunoglobulins M (g/l)						
Day -1	1.44^{a}	0.57^{b}	0.14			
Day 6	1.49^{a}	1.09^{a}	0.14	<0.001	0.751	0.054
Day 19	1.57^{a}	1.27^{a}	0.14			
Reactive Oxygen Metabolites (dROM), CARRU§						
Day -1	469^{b}	428^{b}	23			
Day 6	590^{a}	574^{a}	23	0.217	<0.001	0.662
Day 19	585^{a}	582^{a}	24			
Biological Antioxidant Potential (BAP), μmol/L eq vit C						
Day -1	3467^{bc}	3436c^{a}	85.5			
Day 6	3494^{bc}	3599^{abc}	85.5	0.800	0.002	0.633
Day 19	3847^{a}	3813^{ab}	87.4			
Oxidative Stress Index¶						
Day -1	0.136^{bc}	0.127^{c}	0.006			
Day 6	0.159^{a}	0.151^{ab}	0.006	0.298	<0.001	0.584
Day 19	0.123^{c}	0.124^{c}	0.006			

† Two pig lines differing for their residual feed intake (less efficient High RFI *vs* more efficient Low RFI)

‡ Probability values for the effect of genetic lines, time (days of blood collection) and interaction between lines and time

§CARRU = Carratelli Unit, 1 CARRU = 0.08 mg H_2O_2/100 ml of plasma; BAP = Biological Antioxidant Potential

¶Ratio between dROM and BAP

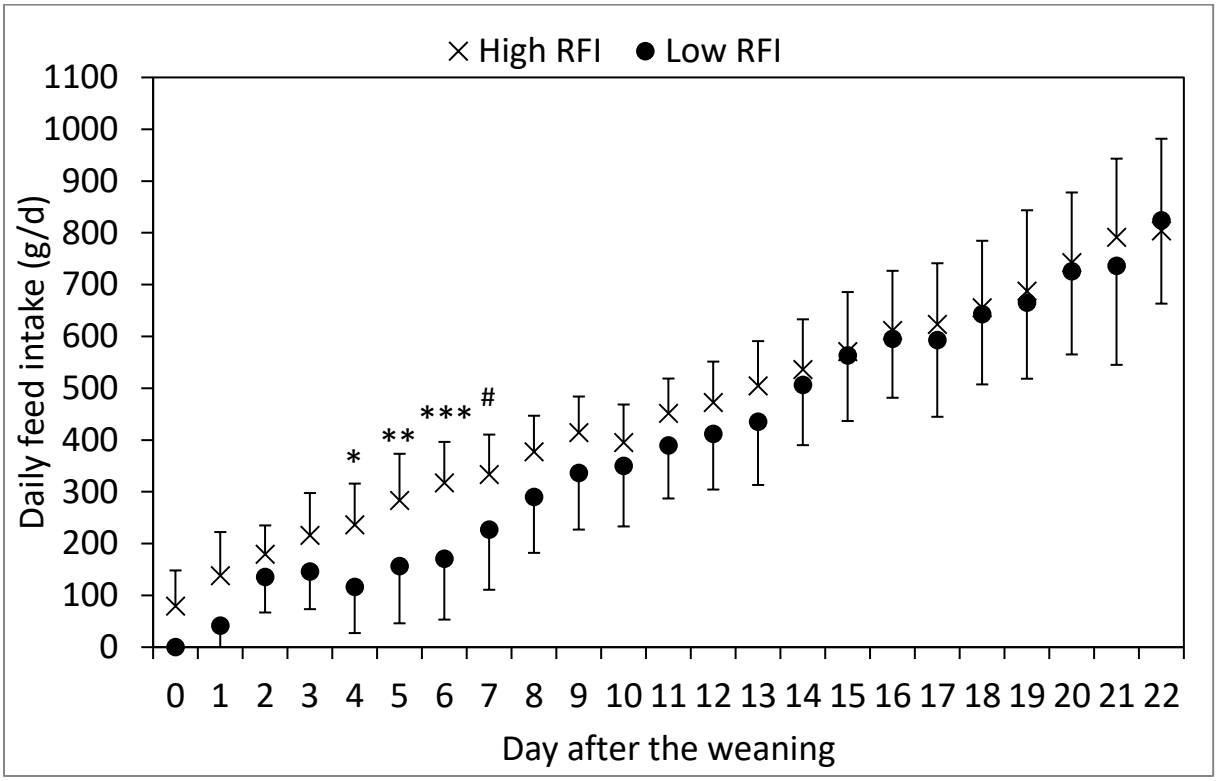
[a,d]Values within a criterion with different superscripts differed significantly at $P<0.05$

x. Figures legends

Figure 1. Average daily feed intake after weaning depending of the pig line (n=34).
Values are lsmeans and standard deviations (n=36).
The two pig lines were divergently selected on residual feed intake (RFI): less efficient High RFI *vs* more efficient Low RFI.
#, *,** and *** indicated trends or significant line differences at the indicated days with P<0.10, 0.05, 0.001 and P<0.001, respectively.

Figure 2. Number of piglets exhibiting diarrhoea after weaning depending of the pig line (n=36).
The two pig lines were divergently selected on residual feed intake (RFI): less efficient High RFI *vs* more efficient Low RFI.
* and ** indicated significant line differences at the indicated days with P <0.05 and P<0.01, respectively

623 Fig 1



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626 Fig 2

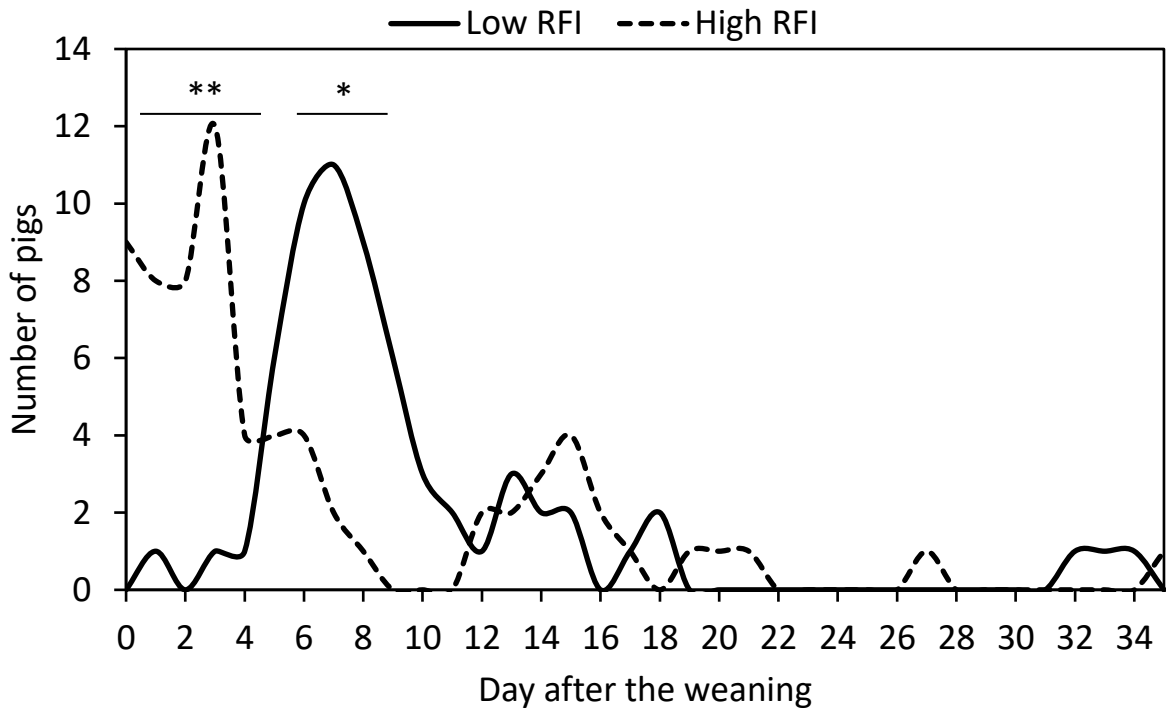

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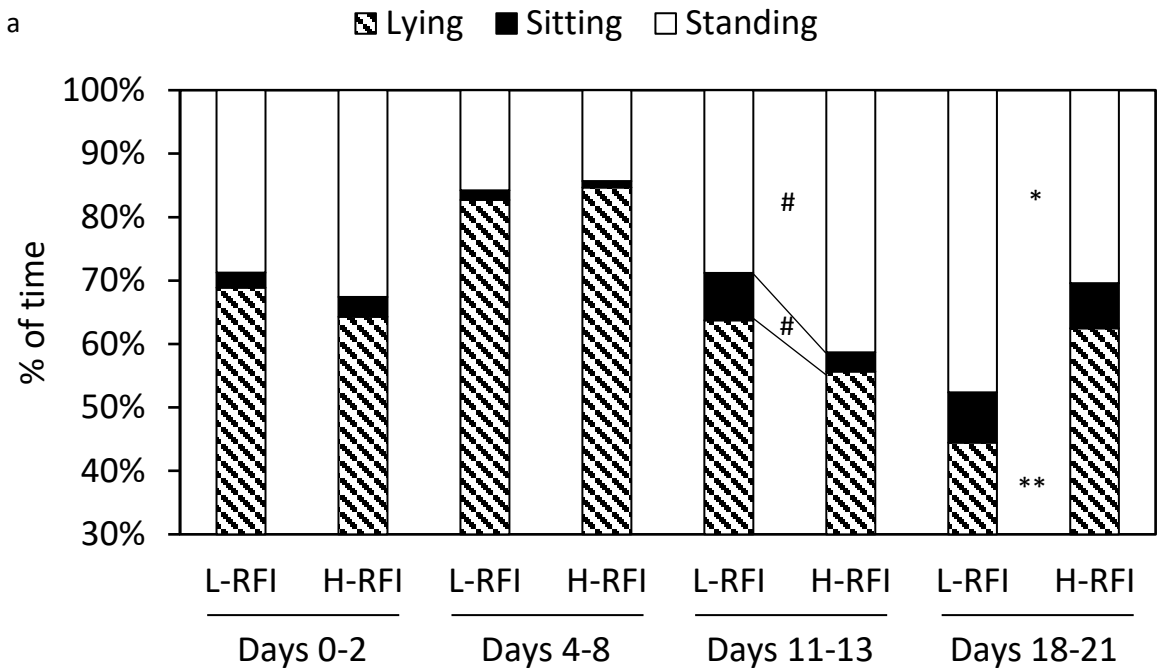
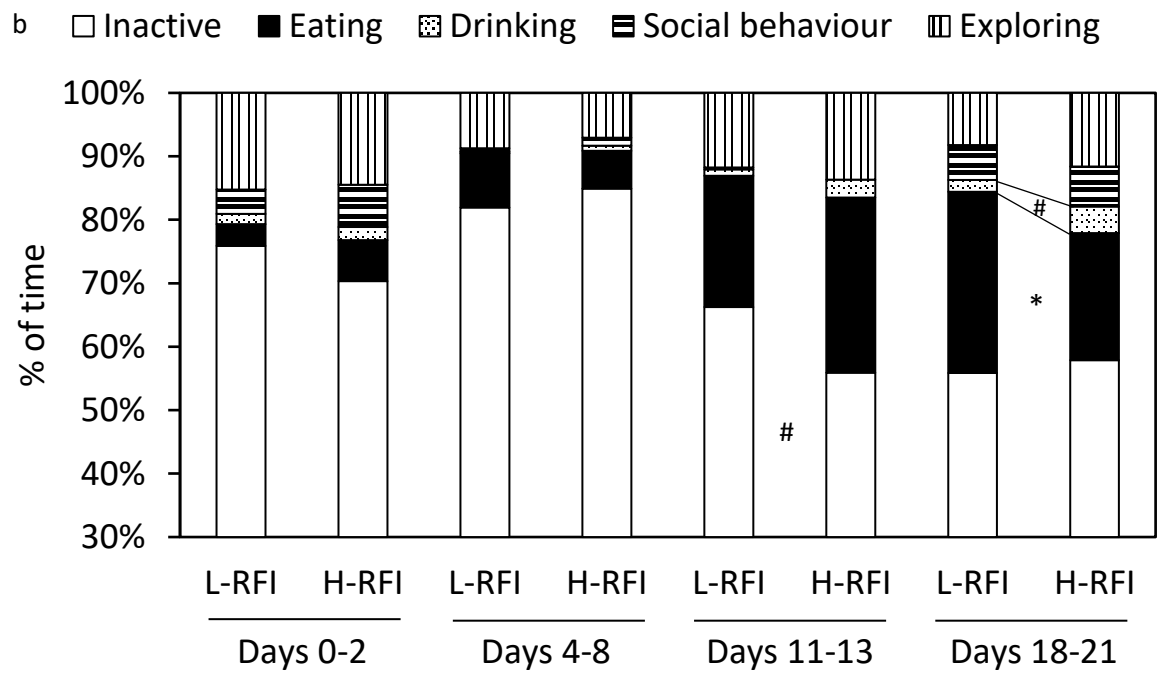
Figure S1. Posture (a) and main activities (b) of pigs after weaning depending of the pig line (N=12).

The two pig lines were divergently selected on residual feed intake (RFI): less efficient High H-RFI *vs* more efficient Low L-RFI.

Data are expressed as percentage of time spent in each posture and activity during each period of observation, based on 30 min scans per day.

#, *,** and *** indicated a trend or line difference at the indicated days with P<0.10, 0.05, 0.001 and P<0.001, respectively.





640 Table S1. Apparent total tract digestibility coefficients (least-square means, n=34) after
641 weaning (day 0) of pigs from two lines divergently selected for residual feed intake (RFI) †

	Line†			P-value‡		
	High RFI	Low RFI	SEM	Line	Time	Line×Time
Dry matter						
Day 6	78.5[b]	71.8[c]	0.87	<0.001	<0.001	<0.001
Day 19	82.4[a]	81.9[a]	0.83			
Organic matter						
Day 6	81.3[b]	75.0[c]	0.83	<0.001	<0.001	<0.001
Day 19	85.4[a]	84.9[a]	0.76			
Minerals						
Day 6	40.0[b]	29.1[c]	1.75	<0.001	<0.001	<0.001
Day 19	60.6[a]	60.7[a]	1.628			
Nitrogen						
Day 6	72.1[b]	59.2[c]	1.80	<0.001	<0.001	<0.001
Day 19	80.3[a]	78.9[a]	1.72			

642 † Two pig lines differing for their residual feed intake (less efficient High RFI *vs* more efficient
643 Low RFI)
644 ‡ Probability values for the effect of genetic lines, time (days of faeces collection) and
645 interaction between lines and time
646 [a,c]Values within a criterion with different superscripts differed significantly at P<0.05
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650 Table S2. Dry matter, ammonia, total concentration and proportions of different volatile fatty
651 acids (VFA, least-square means, n=34) after weaning (day 0) of faeces of pigs from the two
652 lines divergently selected for residual feed intake (RFI) †

	Line†			P-value‡		
	High RFI	Low RFI	SEM	Line	Time	Line×Time
Total VFA, μmol/g faeces						
Day 6	69.23^{b}	63.20^{b}	8.24	0.606	<0.001	0.858
Day 19	178.28^{a}	169.43^{a}	7.56			
Acetate, %						
Day 6	61.37^{ab}	63.38^{a}	0.88	0.106	<0.001	0.057
Day 19	59.95^{b}	58.84^{b}	0.84			
Propionate, %						
Day 6	19.87^{bc}	18.08^{c}	0.55	0.021	<0.001	0.017
Day 19	21.55^{ab}	22.29^{a}	0.52			
Butyrate, %						
Day 6	9.28^{bc}	8.54^{c}	0.43	0.219	<0.001	0.228
Day 19	10.67^{ab}	10.93^{a}	0.41			
Branched chain fatty acid, %						
Day 6	6.76^{a}	7.59^{a}	0.33	0.075	<0.001	0.132
Day 19	5.29^{b}	5.29^{b}	0.31			
Ammonia, μmol/g faeces						
Day 6	18.84^{b}	24.22^{b}	3.49	0.276	<0.001	0.296
Day 19	46.64^{a}	45.02^{a}	3.20			
Ratio VFA by ammonia						
Day 6	4.32^{ab}	2.77^{b}	0.61	0.057	0.006	0.244
Day 19	5.22^{a}	4.93^{a}	0.52			

653 † Two pig lines differing for their residual feed intake (less efficient High RFI *vs* more efficient
654 Low RFI)
655 ‡ Probability values for the effect of genetic lines, time (days of faeces collection) and
656 interaction between lines and time
657 ^{a,c}Values within a criterion with different superscripts differed significantly at P<0.05
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