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1 **Tissue-specific responses of antioxidant pathways to poor hygiene conditions in growing**  
2 **pigs divergently selected for feed efficiency**

3

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14

15 Short title: redox responses to hygiene and production efficiency

16

17 **Abstract**

18 **Background:** Poor hygiene of housing induces a systemic inflammatory response. Because  
19 inflammation and oxidative stress are processes that can sustain each other, the ways pigs are  
20 able to activate their antioxidant defenses are critical for production performance and health  
21 during periods when the immune system is solicited. Selection for production performance  
22 can also influence reactive oxygen species (ROS) production and expression levels of genes  
23 involved in cellular response to oxidative stress in different tissues. To establish the extent by  
24 which poor hygiene and selection for feed efficiency affected redox status, pigs divergently  
25 selected for residual feed intake (RFI) were housed in poor or good hygiene during 6 weeks.  
26 At the end, blood was collected in all pigs, and half of them were killed for tissue sampling.  
27 The remaining pigs were reared in good hygiene conditions during a recovery period of 7-8  
28 weeks.

29 **Results:** At week 6, poor hygiene was associated with a lower total antioxidant capacity  
30 assessed by plasma ferric reducing ability in all pigs, and with greater plasma levels of  
31 hydrogen peroxides in the high RFI pigs (less efficient). Adipose tissue of high RFI pigs  
32 exhibited higher activities of catalase and glutathione reductase, and greater thiobarbituric  
33 acid reactive substances (TBARS) concentrations when compared with the low RFI pigs  
34 (more efficient). Poor hygiene conditions activated the antioxidant enzymes activities  
35 (glutathione reductase, superoxide dismutase and catalase) in adipose tissue of both lines, but  
36 led to higher ROS production by mature adipocytes isolated from the high RFI pigs only. In  
37 liver and muscle, there were only minor changes in antioxidant molecules due to genetics and  
38 hygiene conditions. After the resilience period, adipose tissue of pigs previously challenged  
39 by poor hygiene maintained higher antioxidant enzyme activities, and for the high RFI line,  
40 displayed higher TBARS concentrations.

41

42 **Conclusions:** Pigs selected for improved feed efficiency showed a lower susceptibility to  
43 oxidative stress induced by poor hygiene conditions. This could led to a lower inflammatory  
44 response and less impaired growth when these pigs are facing sanitary challenges during the  
45 production period.

46  
47 **Keywords:** Adipose tissue, Antioxidant enzymes, Feed efficiency, Hygienic challenge,  
48 Oxidative stress

## 49 50 **Background**

51 Poor hygiene and the non-respect of biosecurity rules in commercial farms favor the  
52 occurrence of diseases and subclinical diseases. The housing environment through the  
53 continual exposition to dust, ammonia, microbial and antigens pressure is thus a determinant  
54 of production performance and health of pigs [1,2]. Poor hygiene of housing impairs nutrient  
55 digestibility, feed intake, growth rate, and *in fine*, feed efficiency [3,4]. It also induces a  
56 systemic inflammatory response, so that elevated concentration of haptoglobin, an acute phase  
57 protein found in plasma during inflammation and infection, and higher numbers of  
58 lymphocytes and granulocytes have been observed in blood formula of pigs housed in poor  
59 hygiene conditions [4,5]. Inflammation and oxidative stress are correlated physiological  
60 processes. Indeed, superoxide radicals are formed by the NADPH oxidase from activated  
61 immune cells during inflammatory reactions, while oxidants are activators of the NF- $\kappa$ B  
62 pathway triggering inflammation [6]. Oxidative stress arises when the generation of reactive  
63 oxygen species (ROS) overwhelm the endogenous antioxidant defenses. These defenses rely  
64 on molecules of high (enzymes) and low (glutathione and vitamins) molecular weights, which  
65 can reduce the formation and/or scavenge oxidants and prevent oxidative stress.  
66 Importantly, genetic selection for production performance traits may also affect mitochondrial  
67 functionality, ROS production, and the expression profiles of genes involved in the cellular

68 response to oxidative stress. Among production traits, feed efficiency has received regain  
69 interest because 60-70% of total production costs in farm animals is based on feed. Higher  
70 levels of ROS production and oxidized mitochondrial proteins have been found in muscle of  
71 low feed efficient chickens [7], and the studies also suggest the existence of tissues-specific  
72 regulatory mechanisms on mitochondria protein expressions in these animals. In healthy and  
73 resting pigs, ROS production in mitochondria was higher in *semitendinosus* muscle of the less  
74 efficient pigs selected for high residual feed intake (RFI) when compared with more efficient  
75 pigs selected for low RFI [8]. However, hydrogen peroxides production was similar in the  
76 *longissimus* muscle and liver of both lines [8]. Genes coding for antioxidant enzymes also  
77 exhibited differential expression levels in healthy pigs from the high or low RFI lines, and  
78 differences between lines were tissue-specific [9]. When housed in degraded hygiene  
79 conditions, growth performance and health were more affected in the high RFI pigs than in  
80 the low RFI pigs [5]. We hypothesized that these differences to cope with the inflammation  
81 generated by poor environmental hygiene may be due to line-associated differences in the  
82 ability to cope with oxidative stress. Therefore, the objectives of this study were to investigate  
83 oxidative production and antioxidant capacities of genetically divergent RFI pigs considered  
84 in basal and immune-stimulated conditions. The way different tissues participated to mitigate  
85 oxidative stress when those pigs were facing poor hygiene conditions, was also considered.

86

## 87 **Results**

88 Pigs from two lines divergently selected for low (LRFI) or high (HRFI) residual feed intake  
89 (RFI) were housed either in good or poor hygiene conditions during the first 6 weeks (W)  
90 after their transfer in growing pens (period 1, challenge). Half of these pigs were killed at W6,  
91 while another half were placed in good hygiene conditions (period 2, resilience) until  
92 slaughter at W13 to W14. At each slaughter stage, carcass, digestive tract, lung, and snout

93 were carefully inspected. Any pathological lesions such as pericarditis and pleurisy were  
94 recorded together with the prevalence of pneumonia. Details on the effects of the  
95 environmental hygiene degradation on systemic inflammation, growth performance and the  
96 prevalence and severity of pulmonary lesions at slaughter can be found in our associate  
97 publication [5]. In brief, poor hygiene conditions were associated with a higher prevalence of  
98 pneumonia and lung lesions at the end of period 1 in all pigs, but health of the most efficient  
99 pigs (LRFI) was less impaired.

100

### 101 *Pig performance and body composition*

102 The HRFI pigs grew slower during period 1 (W0 to W6) and were lighter than the LRFI pigs  
103 at the end of this period (Table 1). Although initial body weight (BW) influenced ( $P < 0.01$  to  
104  $P < 0.07$ ) growth performance, covariate analysis attested that the differences between groups  
105 were not solely due to difference in initial BW but were also inherent ( $P < 0.001$ ) to RFI  
106 genetic lines. Importantly, pigs housed in poor hygiene conditions exhibited a lower BW at  
107 the end of period 1 when compared with pigs housed in good conditions. Daily growth rate  
108 from W0 to W6 was reduced due to hygiene degradation in the HRFI pigs, with no significant  
109 difference in the LRFI pigs (Line x Hygiene,  $P = 0.08$ ). In period 2 (W6 to W12), there was  
110 no more difference in daily growth rate between the two lines, but at the end, the HRFI pigs  
111 still tended to be lighter ( $P \leq 0.10$ ) than the LRFI pigs. In addition, pigs housed in poor  
112 hygiene conditions during period 1 were lighter ( $P < 0.01$ ) at the end of the experiment as  
113 compared with pigs never exposed to poor hygiene.

114 At W6 (end of period 1), the relative weights of the perirenal adipose tissue (PRAT) and  
115 *longissimus lumborum* (LL) muscle were lower ( $P < 0.05$ ) in HRFI pigs than in LRFI pigs,  
116 but liver proportion was higher in HRFI pigs than in LRFI pigs (Table 1). The proportion of  
117 dorsal subcutaneous adipose tissue (SCAT) was similar in both lines. Absolute weights of

118 PRAT, SCAT, liver and LL muscle can be found in Additional Table 1. Poor hygiene  
119 conditions decreased the relative weight of PRAT, without any significant changes for SCAT,  
120 LL and liver. At W13-14 (end of period 2), the proportions of adipose tissues and LL muscle  
121 were similar in the four groups of pigs. However, liver proportion was again higher in HRFI  
122 pigs than in LRFI pigs. It was also higher ( $P < 0.05$ ) in pigs housed in poor conditions during  
123 the first period than in pigs never exposed to poor hygiene conditions.

124

#### 125 *Plasma redox status*

126 At W6 (end of period 1), reactive oxygen metabolites (dROM) and total antioxidant activity  
127 estimated by the ferric reducing antioxidant power (FRAP) in plasma did not differ between  
128 the LRFI pigs and HRFI pigs when housed in good hygiene conditions (Table 2). Pigs housed  
129 in poor hygiene conditions had lower FRAP values in plasma than pigs housed in good  
130 conditions. On the opposite, hygiene degradation increased dROM plasma levels in the HRFI  
131 pigs, but had no significant effect in the LRFI pigs (Line x Hygiene,  $P < 0.01$ ). At W13-14  
132 (end of period 2), dROM and FRAP levels in plasma did not differ between the four groups.

133

#### 134 *Tissue lipid content and oxidized lipid products*

135 At W6 (end of period 1), the HRFI pigs had fewer lipids in PRAT ( $P = 0.05$ ) but more lipids  
136 in the LL muscle ( $P = 0.02$ ) when compared with the LRFI pigs (Table 3). Poor hygiene  
137 conditions tended ( $P < 0.10$ ) to reduce lipid content in PRAT, but did not alter total lipid  
138 contents in SCAT, liver and LL muscle as compared with good hygiene. The HRFI pigs  
139 exhibited greater thiobarbituric acid reactive substances (TBARS) levels ( $P < 0.01$ ) in PRAT  
140 when compared with LRFI pigs (Figure 1). There was also a difference in the response of line  
141 to hygiene degradation (Line x Hygiene,  $P = 0.02$ ), so that poor hygiene conditions did not  
142 significantly alter TBARS concentrations in adipose tissue of HRFI pigs but increased their

143 concentrations in LRFI pigs. No differences between the four groups were observed for  
144 TBARS concentrations in liver (Figure 2), except at the beginning of the reaction (T0) when  
145 hepatic TBARS concentration was slightly increased by poor hygiene in the LRFI pigs but  
146 was decreased in the HRFI pigs (Line x Hygiene,  $P < 0.01$ ).

147 At W13-14 (end of period 2), lipid content in PRAT was similar in LRFI and HRFI pigs,  
148 whereas intramuscular lipid content was again greater ( $P < 0.05$ ) in HRFI pigs than in LRFI  
149 pigs (Table 3). At this stage, lipid content in PRAT still tended to be lower in pigs that were  
150 housed in poor conditions during the first period than in pigs always housed in good  
151 conditions. The TBARS levels measured in PRAT were higher in HRFI pigs than in LRFI  
152 pigs (Figure 1). Importantly, the HRFI pigs reared in poor hygiene conditions during period 1  
153 displayed greater TBARS levels in PRAT when compared with HRFI pigs never exposed to  
154 poor hygiene conditions, whereas there were no significant changes due to early hygiene  
155 conditions in the LRFI pigs (Line x Hygiene,  $P = 0.01$ ).

156

### 157 *Antioxidant molecules in tissues*

158 At W6 (end of period 1), the HRFI pigs displayed greater ( $P < 0.05$ ) activity of the  
159 glutathione reductase (GSH-Rx) and catalase (CAT) in PRAT when compared with LRFI pigs  
160 (Table 4). In LL muscle, superoxide dismutase (SOD) activity also tended to be higher ( $P =$   
161  $0.07$ ) in HRFI pigs compared with LRFI pigs. In liver, antioxidant enzymes activities did not  
162 differ between the two lines. At this stage, poor hygiene conditions were associated with  
163 greater activities ( $P < 0.001$  to  $P < 0.10$ ) of the antioxidant enzymes (SOD, GSH-Rx and  
164 CAT) in PRAT when compared with good hygiene. Hygiene had almost no effects on  
165 antioxidant enzymatic defenses in lean tissues, except for SOD activity which was lower ( $P =$   
166  $0.03$ ) in liver of pigs housed in poor conditions. Oxidized (GSSG) and reduced (GSH) forms  
167 of glutathione had similar contents in the liver of all pigs (Table 4).



168 At W13-14 (end of period 2), the HRFI pigs had greater activities of SOD ( $P < 0.01$ ) and  
169 GSH-Rx ( $P = 0.07$ ) in PRAT when compared with the LRFI pigs (Table 4). The HRFI pigs  
170 also had greater GSH-Rx activity ( $P < 0.01$ ) in liver, and there were tendencies for greater  
171 SOD activity ( $P < 0.10$ ) in liver and LL muscle when compared with LRFI pigs. At this time,  
172 pigs housed in poor conditions during period 1 still had activated antioxidant enzymes  
173 activities in PRAT, namely SOD ( $P < 0.001$ ) and GSH-Rx ( $P < 0.01$ ), when compared with  
174 pigs never exposed to poor hygiene. Catalase activity in PRAT was similar in pigs whatever  
175 hygiene conditions of housing in period 1. In liver, early exposure to poor hygiene conditions  
176 was associated with greater SOD activity in HRFI pigs, but lower SOD activity in LRFI pigs  
177 at 13-14 weeks of age (Line x Hygiene,  $P = 0.05$ ).

178

#### 179 *Oxidative enzyme activities in adipose tissue and in vitro ROS production by adipocytes*

180 At W6, the activities of mitochondrial enzymes involved in fatty acid oxidation (beta-  
181 hydroxyacyl-CoA dehydrogenase, HAD) and terminal nutrient oxidation (citrate synthase,  
182 CS) were similar in HRFI and LRFI pigs. Activity levels of these two enzymes were ( $P =$   
183  $0.04$ ) or tended ( $P = 0.07$ ) to be higher in PRAT of pigs housed in poor hygiene conditions  
184 when compared with pigs housed in good conditions (Table 5). At W13-14, there was no  
185 difference in oxidative enzyme activities in PRAT between the four groups of pigs (Table 5).

186 At W6, production of reactive oxygen substrates (ROS) by mature isolated adipocytes was  
187 higher for the HRFI pigs facing poor hygiene conditions than when housed in good conditions  
188 (Figure 3). On the opposite, ROS production by mature adipocytes was lower in LRFI pigs  
189 facing poor hygiene conditions than in LRFI pigs housed in good hygiene conditions (Line x  
190 Hygiene,  $P < 0.01$ ). Addition of LPS or TNF-alpha to the media stimulated ROS production  
191 ( $P < 0.01$ ), except in adipocytes harvested in poor-HRFI pigs where these reagents had no  
192 effects (Figure 3).

193 **Discussion**

194 *Short-term and long-lasting effects of poor hygiene on systemic and tissues redox states*

195 Poor hygiene conditions lowered pig growth rate during the challenge period, and this  
196 reduced the development of visceral adiposity as indicated by the lower relative weight and  
197 lipid content of the perirenal fat. It has been established that poor environmental hygiene  
198 conditions induced a moderate inflammatory state, which was manifested by elevated plasma  
199 concentrations of haptoglobin [4,5,10], an acute phase protein whose concentration increases  
200 in inflammation. Therefore, the difference in adiposity observed in pigs housed in poor  
201 hygiene conditions might be due to an increased production of pro-inflammatory cytokines  
202 such as TNF-alpha, an adipose cytokine known to induce a fat mass loss during acute  
203 inflammatory diseases [11]. Finding greater dROM concentrations and lower total antioxidant  
204 capacity (FRAP) in plasma, the place where oxidative products from diverse origins  
205 accumulate, further indicated the occurrence of oxidative stress in pigs facing poor hygiene  
206 conditions, as defined as the redox imbalance due to excess of oxidants and depletion of  
207 antioxidants [12]. Similarly, an increase in plasma dROM and a decrease in total antioxidant  
208 capacity have been observed in piglets weaned in deteriorated conditions including the lack of  
209 cleaning and disinfection of rooms [13]. Oxidative stress (ROM values) and serum anti-  
210 oxidative capacity are also known to be associated with plasma concentrations of haptoglobin  
211 in young piglets exposed to inflammatory stimuli [14]. Another argument to support the  
212 occurrence of oxidative stress in response to hygiene degradation was the greater activities of  
213 antioxidant enzymes in perirenal adipose tissue of pigs housed in poor hygiene conditions as  
214 compared to pigs housed in good conditions. An up-regulation of antioxidant enzymes in  
215 adipose tissue was similarly observed in the early stages of human obesity, a disease  
216 associated with chronic and low grade inflammatory state [15], before the depletion of the  
217 sources of antioxidant enzymes in the later stages [16,17]. In the present study, pigs facing  
218 poor hygiene conditions also exhibited greater activity levels of mitochondrial enzymes

219 involved in oxidation (citrate synthase) and oxido-reduction process (beta-hydroxyl-acyl-CoA  
220 dehydrogenase) in perirenal adipose tissue, and for high RFI pigs, production of ROS by  
221 adipocytes was higher. Mitochondrial electron transfer for oxidative ATP regeneration is  
222 intrinsically linked to ROS production by the cells [18]. However, there is still a matter of  
223 debate if this mitochondrial metabolic activity can be the cause of the increased ROS  
224 production in adipocytes [19]. After a recovery period in clean conditions, pigs previously  
225 exposed to poor hygiene still tended to exhibit a lower lipid content in perirenal adipose tissue  
226 as compared with pigs never exposed to degraded hygiene, and the activation of antioxidant  
227 enzymes in perirenal adipose tissue was also maintained. This underlined the importance of  
228 early inflammatory events in the regulation of adipose tissue metabolism.

229 Importantly, perirenal fat was identified as the tissue that exhibited most of the changes due to  
230 hygiene conditions, at least when compared with subcutaneous adipose tissue, *longissimus*  
231 muscle and liver. Differences in growth dynamics, metabolism, and immune- and  
232 inflammation-related genes have been previously reported between perirenal and  
233 subcutaneous adipose tissues in pigs [20-22]. The responsiveness of antioxidant enzymes in  
234 adipose tissue has been also underlined in growing piglets exposed to a dietary methionine  
235 deficiency [23], an amino acid with important roles on redox metabolism. Differences  
236 between fat and lean tissues have been identified during the emergence of oxidative stress  
237 associated with obesity, so that antioxidant defenses were affected in adipose tissue but not in  
238 the liver [24]. Finally, numerous studies have pointed the role of adipose tissue physiology on  
239 systemic metabolism and immunity during physio-pathological diseases and deregulated  
240 metabolic states [19]. Adipocytes and adipose-resident immune cells affect each other in a  
241 metabolic-immune interface, so that metabolic features of adipose tissue and their regulations  
242 can provide both local and systemic effects, as shown in various diseases linked to low-grade  
243 inflammation [25]. Adipose tissue was also identified as an extrahepatic source of haptoglobin

244 in humans with excess accumulation of body fat [26]; however, the situation might be  
245 different in young pigs [14]. Taken together, these data indicated that adipose tissue is an  
246 important site bridging energy, redox metabolism, immune and inflammatory stimuli.  
247 Nevertheless, it is noteworthy that at 13-14 weeks of age, there was a trend for a higher liver  
248 weight (relative to BW) in pigs having previously subjected to hygiene degradation during the  
249 first period of growth. Due to contribution of liver to detoxification, synthesis, and release of  
250 biomolecules, this may sign for modifications in protein metabolism due to hygiene  
251 degradation and deserves further studies.

252

253 *Genetic selection for feed efficiency altered redox metabolism in tissues and interacted with*  
254 *their responses to hygiene degradation*

255 In the present study, two pig lines divergently selected for RFI, a measure of feed efficiency,  
256 were compared to test the hypothesis that genetic selection for production traits can affect  
257 redox metabolism in basal and(or) immune-stimulated conditions. Different authors have  
258 identified the response to oxidative stress or to oxygen level, inflammation, immune response  
259 and lipid metabolism as pathways differentially-regulated at the molecular levels between the  
260 two lines when compared in healthy conditions [9,27]. Here, biochemical differences in redox  
261 metabolism between the two RFI lines were revealed in basal and challenged conditions.  
262 Especially, antioxidant enzymes activities were lower and TBARS concentrations were  
263 reduced in perirenal adipose tissue of the low RFI pigs (the most efficient) as compared with  
264 the high RFI pigs (the less efficient). Differences were still significant at weeks 13-14 in  
265 perirenal adipose tissue, and some differences between lines were also observed in the liver  
266 and muscle at this stage. This confirms the existence of tissue-specific differences in the ways  
267 different molecules participating to oxidative/antioxidant pathways were modulated by RFI  
268 divergence, as previously reported in different species [7,8,9]. The liver weight (relative to

269 BW) was also lower in low RFI than in high RFI pigs at the two periods (challenge and  
270 resilience). This observation fits with the difference reported between these lines at different  
271 stages of growth [9,28], and may be associated with difference in catabolic pathway activities  
272 between the two lines [28]. Importantly, the two lines responded somewhat differently to the  
273 hygienic challenge, so that not only growth rate was more affected in the high RFI pigs, but  
274 plasma dROM concentrations also increased specifically in these pigs when exposed to poor  
275 hygiene conditions. It has been reported that average daily gain, feed intake, feed efficiency  
276 and plasma haptoglobin concentrations were more affected by hygiene degradation in the high  
277 RFI pigs [5]. Adipocyte ROS production in response to pro-inflammatory situations were also  
278 different between the two RFI lines. First, adipocytes isolated from high RFI pigs exposed to  
279 poor hygiene produced more ROS than the other groups. Moreover, addition of TNF-alpha or  
280 liposaccharides (LPS) increased ROS production in all pigs, except in high RFI pigs housed in  
281 poor hygiene conditions. The increase in adipocyte ROS production due to pro-inflammatory  
282 reagents was in accordance to what is observed in murine 3T3-L1 adipocytes [29,30].  
283 Because adipose cells from the high RFI pigs housed in poor hygiene conditions produced  
284 ROS at very high levels when cultured in the control medium, these cells might be  
285 desensitized to any re-stimulating inflammatory conditions such as TNF-alpha and LPS. After  
286 a recovery period, TBARS concentrations in perirenal adipose tissue were affected by early  
287 hygiene conditions in the high RFI pigs only, suggesting a longer lasting susceptibility to  
288 oxidative stress for the less efficient pigs. In agreement, long lasting effects of poor hygiene  
289 on lesions scoring (pleurisy, pericarditis) were greater in high RFI pigs than in low RFI pigs  
290 [5]. Altogether, these data support the view that pigs selected for low RFI may be less prone  
291 to oxidative stress as compared with high RFI pigs [8]. Because RFI is considered as a  
292 biological estimate of the net efficiency in nutrient utilization, this might be related to a better  
293 efficiency of mitochondria metabolism and changes in the mitoproteome [31] in low RFI pigs.

294

## 295 **Conclusions**

296 Differences for redox metabolism in the perirenal adipose in both basal and challenged  
297 conditions were revealed between two porcine lines divergently selected for feed efficiency  
298 tissue. A lower susceptibility to oxidative stress was suggested for pigs selected for low  
299 residual feed intake, an observation that corroborates the lower inflammatory response and  
300 reduced growth disturbance previously reported in those pigs when facing poor hygiene  
301 conditions. This study demonstrates that selection for improved feed efficiency can modulate  
302 physiological systems important for adaptation and health. This could be of importance for  
303 pigs in production farms to limit economic losses and improve welfare. Among tissues,  
304 perirenal adipose tissue features highly responded to hygiene degradation, suggesting that this  
305 tissue could play an important role in the management of oxidative stress when pigs are facing  
306 pro-inflammatory environmental conditions.

307

## 308 **Methods**

### 309 **Ethics**

310 Animals were cared in compliance with the French directive on animal experimentation, and  
311 they were killed in compliance with national regulations and according to procedures  
312 approved by the French Veterinary Services at INRA experimental facilities. The study was  
313 submitted to and approved by our institutional ethics committee (Comité Rennais d'Ethique  
314 en matière d'Expérimentation Animale, CREEA, Rennes, France) and authorized by the  
315 French Ministry of National Education, Higher Education and Research.

### 316 **Animals**

317 The trial included 71 Large-White pigs produced from the 8th generation of a divergent  
318 genetic selection for RFI [32]. The RFI represents the difference between observed and

319 expected feed intake of an animal based on the estimated requirements for its maintenance  
320 and growth. The RFI criterion was calculated as follows:

$$321 \text{ RFI} = \text{ADFI} - (1.24 \times \text{ADG}) - (31.9 \times \text{BFT}),$$

322 where ADFI is the average daily feed intake (g/day) between 35 and 95 kg BW, ADG is the  
323 average daily gain (g/day) during the same period, and BFT is the backfat thickness (mm)  
324 used as a surrogate of adiposity at 95 kg BW. The objectives of the selection were keeping  
325 ADG and BFT constant between the two lines [32]. For selection, the RFI of boars was  
326 computed using their own records for ADFI, ADG and BFT, and taking into account the fixed  
327 effects of contemporary group and pen size. The  $R^2$  of the model to compute the predicted  
328 feed intake of P1 animals was 0.66 [33]. As compared with pigs selected for high RFI (HRFI),  
329 pigs selected for low RFI (LRFI) have an improved gain-to-feed ratio, which is a widely used  
330 indicator of feed efficiency.

331 All pigs were born in the INRA experimental facilities (Unité Experimentale Porcs de Rennes,  
332 35590 Saint-Gilles, France) and weaned at 4 weeks. The disease status of the RFI herd was  
333 negative for *Actinobacillus* and porcine reproductive and respiratory syndrome virus (PRRS),  
334 but it was positive to porcine respiratory Coronavirus and *Mycoplasma hyopneumoniae*. The  
335 experiment began when pigs reached 12 weeks of age, hereafter referred as Week 0 [W0].  
336 The experiment was divided in two periods: a challenge period (Period 1) and a recovery  
337 period (Period 2). The challenge period (Period 1) started on W0 and lasted 6 weeks. Pairs of  
338 littermates (same sex: male or female) were constituted within each RFI line (n = 40 for low  
339 RFI and n = 32 for high RFI pigs). Within each pair, animals were assigned to one of the two  
340 groups: good or poor hygiene conditions. One animal died from acute diarrhoea soon after the  
341 beginning of the experiment. Therefore, a total of 36 males and 35 females was finally  
342 considered: 8 males and 7 females for HRFI pigs housed in good conditions, 8 males and 8  
343 females for HRFI pigs housed on poor conditions, 10 males and 10 females for LRFI housed

344 in good conditions, and 10 males and 10 females for LRFI pigs housed in poor conditions.  
345 Housing procedures were derived from Le Floch and colleagues [4] and details on how poor  
346 or good hygiene conditions were established are available in our associate publication [5].  
347 Briefly, the poor conditions consisted of no cleaning nor sanitation of the room after previous  
348 occupation by non-experimental pigs, a decreased aeration rate and no biosecurity precaution;  
349 experimental pigs in poor housing conditions were also mixed with non-experimental pigs  
350 during the challenge period. Conversely, the aeration rate and temperature were optimal in  
351 clean conditions, and the room was disinfected; strict biosecurity precautions were applied,  
352 including no mixing of the experimental pigs with non-experimental pigs. At W6, half of the  
353 pigs (pairs of pigs balanced for the sex, line and hygiene conditions) was killed. The recovery  
354 period (Period 2) lasted from W6 to W13-14. During this period, all the remaining pigs were  
355 housed in good conditions until slaughter.

356 Throughout the experimental period, pigs were housed in individual pens (85×265 cm) on  
357 concrete floor. They were fed ad libitum a standard growing diet used for selection (94.7 kJ  
358 digestible energy; tryptophane/lysine 0.2; lysine 8.3 g/kg; dry matter 87.38%; starch 44.17%;  
359 neutral detergent fibre 15.7%; acid detergent fibre 5.6%; acid detergent lignin 1.6%; fat  
360 3.14%; wheat 32.2%; barley 30%; maize 15%; soya bean 7%; bran 5%). They had free access  
361 to water. Pigs were weighed after an overnight fast at W0, W6, W12, and just before slaughter  
362 at W13 or W14. Feed consumption was estimated weekly as the difference between allocated  
363 feed minus feed refusals.

#### 364 **Blood and tissues collection**

365 The day before slaughter at W6 or W13-14, and after an overnight fast, blood was collected  
366 between 08:00h and 10:00h by jugular punctures in heparin (9 ml) or ethylene-diamine-tetra-  
367 acetic acid (EDTA) vacutainers tubes (4 and 9 ml). Samples were kept on ice, until  
368 centrifugation (2,500 x *g*, 4°C, 15 min), and plasma was aliquoted and kept at -80°C until  
369 analyses. In the next morning, the pigs were weighed. Then, they were transported to the



370 experimental slaughterhouse (Unité Experimentale Porcs de Rennes, INRA, Saint Gilles,  
371 France), to be euthanatized by electrical stunning and exsanguination. Animals of each group  
372 were alternatively killed. The liver and the entire PRAT were removed and weighed. A sample  
373 (50 to 100 g) of the LL muscle was also collected at the level of the last rib from the right side  
374 of the carcass within 30 min after pig death. Tissue samples were cut into small pieces  
375 (approximately 0.5 x 0.5 x 0.5 cm), snap frozen in liquid nitrogen, and stored at -76°C until  
376 metabolic analyses. Portions (100 g) of liver, PRAT and LL muscle were also kept at -20°C in  
377 vacuum plastic bags; liver and LL were subsequently freeze-dried and stored again at -20°C.  
378 For pigs at W6, about 15 g of adipose tissue was also put in warm Krebs-Ringer buffer before  
379 digestion for cellular isolation. The day after slaughter, dorsal SCAT was collected from the  
380 left side of the carcass and weighed; a portion (100 g) was kept at -20°C in a vacuum plastic  
381 bag. The LL muscle was also dissected from the loin of the same carcass side, and weighed.  
382 Organ and tissue weights were expressed relative to BW.

### 383 **Total lipid content and TBARS index**

384 Sub-samples of frozen (PRAT and SCAT) and freeze-dried (LL and liver) tissues were ground  
385 in a cutter mill (Grindomix GM200, Retsch, Newton, PA). In PRAT and SCAT (500 mg each)  
386 and in LL muscle (approximately 800 mg), total lipid content was determined by the  
387 application of supercritical CO<sub>2</sub> and solvent extraction [34] with an automatic system (Leco  
388 TFE 2000 Instrument, Leco, St. Joseph, MI). In liver (approximately 2.5 g of sample), lipid  
389 content was determined using a chloroform and methanol (2:1) extraction procedure [35].  
390 Results were expressed in gram of lipids per 100 g of wet tissue weight.

391 The TBARS concentration was determined in PRAT and liver samples submitted to different  
392 incubation times (0, 60, 120, 180 and 240 min) at 37°C, with a forced chemical oxidation  
393 induced by iron trichloride and sodium ascorbate [36]. The TBARS concentration was  
394 expressed in nmoles of malondialdehyde (MDA) produced per g of tissue.

### 395 **Antioxidant enzyme activities**

396 The activities of CAT, total SOD and GSH-Rx were determined in liver, PRAT and LL  
397 (approximately 100 mg each) after tissue homogenization in 0.8 mL ice-cold sucrose buffer  
398 (0.5 M) containing 0.05 M Tris-HCl and 1 mM EDTA (pH 7.4). The CAT activity was  
399 measured by spectrometry at 240 nm using an Uvikon Bio-Tek apparatus (Secomam, Alès,  
400 France) following the decrease in H<sub>2</sub>O<sub>2</sub> concentration at 25°C [37]. Total SOD activity was  
401 measured at 450 nm by the inhibition of the xanthine/xanthine oxidase-mediated oxidation of  
402 cytochrome-c, using a dedicated kit (19160 SOD; Sigma Aldrich, St. Louis, MO, USA) with a  
403 SOD standard from bovine erythrocytes (S7571-15KU; Sigma Aldrich) and a microplate  
404 reader (Thermo-Labsystems, Franklin, MA). Activity of GSH-Rx was assessed at 37°C by  
405 following NADPH decrease at 340 nm absorbance [38], using a KoneLab 20i analyzer  
406 (Thermo Scientific, Cergy Pontoise, France). All enzyme activities were expressed as  
407 units/min and per g of tissue wet weight.

### 408 **Oxidative enzyme activities**

409 Activities of HAD and CS were measured in PRAT. Briefly, adipose samples (300 mg each)  
410 were homogenized in phosphate buffer (pH 7.4) containing 2 mM EDTA. Mixtures were  
411 sonicated (60 s, 50 Hz; Bioblock scientific, Illkirch, France), centrifuged (13 min at 1,500g at  
412 4°C; Mikro 200R Hettich, Sigma-Aldrich, St. Louis, MO, USA), and supernatants were stored  
413 on ice. Activities were immediately assayed by spectrometry in the KoneLab analyzer at 30°C  
414 following dedicated methods, at 340 nm absorbance for HAD [39] and at 405 nm for CS [40],  
415 respectively, and expressed as µmole/min/g tissue.

### 416 **Glutathione content**

417 Contents in GSH and GSSG were enzymatically analyzed in liver [41]. Briefly, samples (100  
418 mg) were homogenized in ice-cold buffer with 2 mL 5-sulfosalicylic acid (5% w/v) and  
419 centrifuged for 5 min at 10 000g at 4°C. The supernatants were collected and analyzed using

420 the Glutathione Assay Kit (7511-100-K; Trevigen, Gaithersburg, MD, USA) and the  
421 microplate reader. Contents are expressed as pmol of glutathione per well.

#### 422 **Plasma redox**

423 The dROM contents were measured in plasma samples (250  $\mu$ L) on the Konelab analyzer by  
424 the dROM test (Diacron, Grosseto, Italy), described as a simple assay for analyzing the total  
425 amount of hydrogen peroxides [42]. Total antioxidant activity in plasma was estimated by  
426 FRAP method [43], which was based on the reduction of the 2,4,6-tripyridyl-s-triazine  
427 (TPTZ) with ferric chloride hexahydrate producing blue ferrous complexes producing intense  
428 blue color ferrous complexes. The final absorbance was measured at 595 nm after 30 min  
429 incubation at 37°C in the darkness [44], using a microplate reader (Thermo-labsystems,  
430 Franklin, MA). Measurements were performed in triplicates and results were expressed as  
431 molar Trolox equivalents antioxidant capacity (TEAC) per L.

#### 432 **ROS production by adipocytes**

433 From a subset of pigs slaughtered at W6 (n = 8 per group), mature adipocytes were isolated  
434 following standard procedures [45], by digestion of adipose tissue samples (15 g) in HEPES  
435 buffer (2 mL/g of tissue) containing 2% BSA and 2 mg/mL collagenase A. A fivefold excess  
436 of buffer free of collagenase was added, before cells were centrifuged at 400  $\times$  g for 10 min.  
437 Floating adipocytes were immediately placed into culture media with or without TNF-alpha  
438 (1ng/mL; Invitrogen-Life Technologies, Saint-Aubin, France) or Gram-negative bacterial  
439 lipopolysaccharides (LPS; 1mg/mL; *Escherichia coli* O55:B5, Sigma-Aldrich, St-Louis, MO)  
440 to induce an inflammatory response. The production of ROS by the cells in the different  
441 culture conditions was determined by a fluorimetric assay after 90 min of incubation with 5  
442  $\mu$ M of DHCF [46]. The fluorescence was measured with a multi-detection microplate reader  
443 (Mithras, LB 940, Berthold Technologies, Bad Wildbad, Germany) using an excitation and  
444 emission light at 485 nm and 535 nm, respectively. Samples were also incubated in the

445 presence of 50 U of PEG-SOD, which was used as negative control to assess the specificity of  
446 the assay.

#### 447 **Statistical analyses**

448 Statistical analyses were carried out separately for each period (period 1: challenge; period 2:  
449 resilience) using the SAS software (SAS Institute, Cary NC, New-York), and considering pig  
450 as the experimental unit. For all traits except adipocyte ROS production and TBARS  
451 concentrations, GLM procedures were used to analyze data with models including hygiene  
452 conditions (good or poor), line (LRFI or HRFI), and the interaction between hygiene  
453 conditions and line as the main effects. How initial BW may also influence final BW and  
454 ADG was tested using initial BW as a covariable in the models. For ROS production, the  
455 model included hygiene conditions (poor or good), line (LRFI or HRFI), and presence of LPS  
456 (yes or no) or TNF-alpha (yes or no), and all the interactions between the main factors. For  
457 TBARS concentrations, a MIXED model was used with hygiene conditions, line and the  
458 interaction between hygiene conditions and line as the main effects, and a repeated statement  
459 [47] to indicate that data are correlated on the same animal during the forced oxidation  
460 kinetics. Interaction effects between line or hygiene conditions and time were also tested but  
461 were not significant.  $P \leq 0.05$  was considered significant, and  $0.05 < P \leq 0.10$  was discussed  
462 as a trend.

463

#### 464 **List of abbreviations**

465 **ADG:** average daily gain

466 **BFT:** backfat thickness

467 **BW:** body weight

468 **CS:** Citrate synthase

469 **dROM:** diacron reactive oxygen metabolites

- 470 **EDTA:** ethylene-diamine-tetra-acetic acid
- 471 **FRAP:** ferric reducing ability of plasma
- 472 **GGSG:** oxidized form of glutathione
- 473 **GSH:** reduced form of glutathione
- 474 **GSH-Rx:** glutathione reductase
- 475 **HAD:** beta-hydroxy-acyl-CoA-dehydrogenase
- 476 **HRFI:** high residual feed intake
- 477 **LL:** *longissimus lumborum* muscle
- 478 **LPS:** bacterial liposaccharides
- 479 **LRFI:** low residual feed intake
- 480 **MDA:** malondialdehydes
- 481 **MSE:** mean standard error
- 482 **NADPH:** nicotinamide adenine dinucleotide phosphate, reduced form
- 483 **PRAT:** perirenal adipose tissue
- 484 **RFI:** residual feed intake
- 485 **ROS:** reactive oxygen species
- 486 **SCAT:** subcutaneous backfat adipose tissue
- 487 **SOD:** superoxide dismutase
- 488 **TBARS:** thiobarbituric acid reactive substances
- 489 **TNF-alpha:** tumor necrosis factor-alpha
- 490 **W:** week
- 491
- 492 **Declarations**
- 493 ***Ethics approval and consent to participate***
- 494 Animals were cared in compliance with the French directive on animal experimentation
- 495 (<https://www.legifrance.gouv.fr/eli/decret/2013/2/1/2013-118/jo/texte>). The protocol was

496 submitted and approved by the Ethical Committee in Animal Experiment N°7  
497 (<http://ethique.ipbs.fr/creeapresent.html>; Comité Rennais d’Ethique en matière  
498 d’Expérimentation Animale, CREEA, Rennes, France). The formal authorization was then  
499 delivered from the French Ministry of National Education, Higher Education and Research,  
500 following the written notification of the ethics committee. All participants have a valid  
501 agreement to experiment on living animals  
502 (<https://www.legifrance.gouv.fr/eli/arrete/2013/2/1/AGR1238729A/jo/texte>). Pigs were  
503 killed in compliance with national regulations and according to procedures approved by the  
504 French Veterinary Services at INRA experimental facilities.

505

#### 506 ***Consent to publish***

507 Not applicable.

508

#### 509 ***Availability of data and material***

510 Individual data used/analysed in this study are available from the corresponding author on  
511 reasonable request.

512

#### 513 ***Competing interests***

514 The authors declare that they have no competing interests.

515

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524 conclusions.

525

### 526 ***Authors' contributions***

527 NLF: conceived the project and its animal design. FG: conceived and coordinated the study  
528 on oxidant and antioxidants in tissues; MHP: conceived and realized cellular investigations;  
529 KS and EM: generated data on plasma redox status. KS: generated tissue biochemical data.  
530 KS, FG, NLF, EM, and MHP: participated to sample collection; FG: performed statistics,  
531 analysed the data and interpreted the results; FG: drafted the manuscript. All authors helped to  
532 draft the manuscript and read and approved the final version.

533

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670

## 671 **Figure Legends**

672 **Fig. 1.** Effects of RFI line and hygiene conditions on lipid byproducts in adipose tissue

673 Pigs from two genetic lines divergently selected for low (LRFI) or high (HRFI) RFI were  
674 housed in good or poor hygiene conditions during 6 weeks (period 1, challenge). Half of these  
675 pigs were killed at week 6, whereas another half were placed in good hygiene conditions until  
676 slaughter at weeks 13 to 14 (period 2, recovery). Thiobarbituric reactive substances (TBARS)  
677 were measured in perirenal adipose tissue (PRAT) at different time points along forced  
678 chemical oxidation, and expressed as malondialdehydes (MDA) concentration (nM/g tissue).  
679 Effects of genetic line, hygiene conditions and their interaction (LxH) were tested for repeated  
680 measures data of TBARS along the kinetics. \*\*\* $P < 0.001$ ; \*\*  $P < 0.01$ ; \* $P \leq 0.05$ . Dashed  
681 lines are used for poor hygiene conditions, and continuous lines are used for good hygiene  
682 conditions; red color is used for high RFI pigs and blue colour is used for low RFI pigs.

683

684 **Fig. 2.** Effects of RFI line and hygiene conditions on lipid byproducts in the liver  
685 Pigs selected for low (LRFI) or high (HRFI) residual feed intake (RFI) were housed either in  
686 good or poor hygiene conditions during 6 weeks. Thiobarbituric reactive substances (TBARS)  
687 in the liver were measured at different time points along a forced chemical oxidation kinetics,  
688 and expressed as malondialdehydes (MDA) concentrations (nM/g tissue). Effects of line,  
689 hygiene conditions and their interaction (LxH) were tested for repeated measures data of  
690 TBARS along the kinetics. LxH: interaction effect between genetic line (L) and hygiene  
691 conditions (H):  $^{**}P = 0.01$ . Dashed lines are used for poor hygiene conditions, and  
692 continuous lines are used for good hygiene conditions; red color is used for high RFI pigs and  
693 blue color is used for low RFI pigs.

694

695 **Fig. 3.** Reactive oxygen species (ROS) production in isolated mature adipocytes  
696 Reactive oxygen species (ROS) production was determined in adipose cells isolated in pigs  
697 selected for low (LRFI) or high (HRFI) RFI and housed in good or poor hygiene conditions  
698 during 6 weeks. A fluorimetric assay using 2,7-dihydrochlorofluorescein (DHCF) was used to  
699 estimate ROS production into cells. Adipocytes were cultured in media with or without  
700 bacterial lipopolysaccharides (LPS: 1 mg/mL) or tumor necrosis factor (TNF-alpha: 1  
701 ng/mL). A fluorimetric assay using 2,7-dihydrochlorofluorescein (DHCF) was used to  
702 estimate ROS production into cells. Data are expressed as mean  $\pm$  S.E.M. (n = 8 pigs per  
703 group). LxH: interaction effect between genetic line (L) and hygiene conditions (H); LPS:  
704 effect of LPS; TNF-alpha: effect of TNF-alpha.  $^{**}P < 0.01$ . Hatched charts are used for poor  
705 hygiene conditions, and full charts are used for good hygiene conditions; red color is used for  
706 high RFI pigs and blue color is used for low RFI pigs.

707

708 **Additional files:**

709 **Additional file 1:** Effects of RFI line and hygiene conditions on tissue weights of pigs.

710 Weights of adipose tissues collected at the perirenal (PRAT) or subcutaneous (SCAT)  
711 locations, loin skeletal muscle (LL: *longissimus lumborum*) and liver in pigs at the two time  
712 points (week 6 and week 13-14) are given in this additional Table.

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715 **Table 1 Effects of RFI line and hygiene conditions on growth performance of pigs**

Line	Low RFI	High RFI	P values						
Hygiene	Good	Poor	Good	Poor	MSE	Line	Hygiene	LxH	
<b>Period 1 (n = 71)</b>									
BW W0	27.9	28.0	24.7	25.0	3.2	<b>&lt;0.001</b>	0.80	0.93	
BW W6	62.5	58.6	55.6	48.1	6.2	<b>&lt;0.001</b>	<b>0.006</b>	0.23	
ADG	808c	717bc	762b	537a	115	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.08</b>	
Proportions of tissues									
SCAT	5.0	4.7	5.0	4.7	0.7	0.91	0.18	0.96	
PRAT	0.56	0.48	0.48	0.39	0.11	<b>0.02</b>	<b>0.02</b>	0.99	
Liver	2.3	2.3	2.6	2.7	0.2	<b>0.005</b>	0.56	0.71	
LL	5.5bc	5.8c	5.3ab	5.1a	0.4	<b>0.002</b>	0.75	<b>0.07</b>	
<b>Period 2 (n = 35)</b>									
BW W12	102.4	90.8	98.8	81.1	10.0	<b>0.06</b>	<b>0.002</b>	0.38	
End BW	111.0	100.2	110.2	90.1	9.4	<b>0.10</b>	<b>&lt;0.001</b>	0.17	
ADG	888	774	959	832	147	0.21	<b>0.02</b>	0.88	
Proportions of tissues									
SCAT	6.8	6.2	6.7	5.8	1.5	0.65	0.14	0.71	
PRAT	0.86	0.87	0.92	0.67	0.22	0.31	0.15	0.11	
Liver	1.61	1.72	1.98	2.11	0.17	<b>&lt;0.001</b>	<b>0.04</b>	0.90	
LL	5.7	5.6	5.5	5.3	0.6	0.20	0.39	0.53	

716 Pigs from two lines divergently selected for low (LRFI) or high (HRFI) residual feed intake  
717 (RFI) were housed either in good or poor hygiene conditions during the first 6 weeks (W)  
718 after their transfer in growing-finishing pens (period 1, challenge). Half of these pigs were  
719 killed at week 6 (W6: n = 20 LRFI pigs in good conditions, n = 20 HRFI pigs in good  
720 conditions, n = 15 LRFI pigs in poor conditions, n = 16 HRFI pigs in poor conditions).  
721 Another half were placed in good hygiene conditions until slaughter at weeks 13 or 14 (period  
722 2, recovery: n = 10 LRFI pigs from good conditions, n = 10 HRFI pigs from good conditions,  
723 n = 7 LRFI pigs from poor conditions, n = 8 HRFI pigs from poor conditions). Body weight  
724 (BW, kg) at week 0 (W0), 12 (W12) and just before slaughter (end BW) were individually  
725 assessed. Average daily gain (ADG, g/d) was calculated during the two periods. Tissues (LL:  
726 *longissimus lumborum* muscle; PRAT: perirenal adipose tissue, SCAT: subcutaneous adipose  
727 tissue) were weighed at slaughter, and expressed in proportion to BW. Letters (a,b,c) were  
728 added in case of interaction (LxH;  $P < 0.10$ ) between hygiene (H) and RFI line (L), and  
729 means sharing a common letter did not differ. MSE: root mean standard error of the statistical  
730 model. Bold face highlights significant differences ( $P \leq 0.05$ ) between treatments, and when  
731 italicized, this denotes a trend ( $0.05 < P \leq 0.10$ ).

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734 **Table 2 Effects of RFI line and hygiene conditions on redox status in plasma of pigs**

Line	RFI		P values					
	Low	High						
Hygiene	Good	Poor	Good	Poor	MSE	Line	Hygiene	LxH
<b>Week 6 (n = 71)</b>								
dROM	987ab	1023b	857a	1200c	233	0.68	<b>0.001</b>	<b>0.008</b>
FRAP	203	192	210	185	22	0.95	<b>0.002</b>	0.18
<b>Week 13-14 (n = 35)</b>								
dROM	1146	990	1045	1102	194	0.94	0.50	0.14
FRAP	202	232	202	207	30	0.71	0.31	0.11

735 Pigs from two lines divergently selected for low (LRFI) or high (HRFI) residual feed intake  
736 (RFI= were housed either in good or poor hygiene conditions during the first 6 weeks after  
737 their transfer in growing-finishing pens (period 1). Half of these pigs were killed at week 6,  
738 whereas another half were placed in good hygiene conditions until slaughter at weeks 13 or 14  
739 (period 2). Blood was sampled from the jugular in living pigs at the end points of the two  
740 periods. Systemic reactive oxygen metabolites (dROM) were expressed as Carratelli Unit (1  
741 CARRU = 0.08 mg H<sub>2</sub>O<sub>2</sub>/100 mL). Ferric reducing ability of plasma (FRAP), a measure of its  
742 antioxidant power, was expressed as molar Trolox equivalents per L. Letters (a,b,c) were  
743 added in case of interaction (LxH; *P* < 0.10) between hygiene (H) and RFI line (L), and  
744 means sharing a common letter did not differ. MSE: root mean standard error of the statistical  
745 model. Bold face highlights significant differences (0.05 < *P* ≤ 0.05) between treatments.

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749 **Table 3 Effects of RFI line and hygiene of housing conditions on tissue lipid content in**  
 750 **growing pigs**

Line	Low		High		<i>P</i> values				
	RFI		RFI		MSE	Line	Hygiene	LxH	
Hygiene	Good	Poor	Good	Poor					
<b>Week 6 (n = 36)</b>									
SCAT	61.7	59.2	60.0	59.1	7.2	0.71	0.48	0.73	
PRAT	71.2	66.3	65.9	59.6	9.1	<b>0.05</b>	<b>0.08</b>	0.83	
Liver	3.2	3.5	3.3	3.5	0.4	0.60	0.11	0.82	
LL	0.81	0.70	0.95	1.06	0.30	<b>0.02</b>	0.99	0.30	
<b>Week 13-14 (n = 35)</b>									
SCAT	77.2	70.7	69.4	67.5	5.8	0.12	0.45	0.96	
PRAT	81.9	79.9	83.8	79.0	5.1	0.83	<b>0.07</b>	0.48	
Liver	3.5	4.0	3.7	4.1	0.8	0.53	0.12	0.94	
LL	0.88	0.99	1.36	1.23	0.35	<b>0.002</b>	0.33	0.52	

751 Pigs from two genetic lines divergently selected for low (LRFI) or high (HRFI) RFI were  
 752 housed either in good or poor hygiene conditions during the first 6 weeks after their transfer  
 753 in growing-finishing pens (period 1). Half of these pigs were killed at week 6, whereas  
 754 another half were placed in good hygiene conditions until slaughter at weeks 13 or 14 (period  
 755 2). Subcutaneous (SCAT) and perirenal (PRAT) adipose tissues, liver and *longissimus*  
 756 *lumborum* (LL) muscle were sampled in pigs at the end points of the two periods. Lipid  
 757 content was expressed in gram per 100 g of wet tissue. LxH: interaction between hygiene (H)  
 758 and RFI line (L). MSE: root mean standard error of the statistical model. Bold face highlights  
 759 significant differences ( $P \leq 0.05$ ) between treatments, and when italicized, this denotes a trend  
 760 ( $0.05 < P \leq 0.10$ ).

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763 **Table 4 Effects of RFI line and hygiene of housing conditions on antioxidant enzymes**  
 764 **and glutathione content in tissues of growing pigs**

Line	Low RFI		High RFI		P values				
	Good	Poor	Good	Poor	MSE	Line	Hygiene	LxH	
<b>Week 6 (n = 36)</b>									
SOD									
PRAT	81.4	109.4	99.5	104.8	26.1	0.44	<b>0.06</b>	0.17	
Liver	2727	2289	2929	2520	551	0.25	<b>0.03</b>	0.94	
LL	203	201	224	218	29.8	<b>0.07</b>	0.68	0.82	
CAT									
PRAT	1002	1299	1169	1500	258	<b>0.04</b>	<b>0.001</b>	0.84	
Liver	72.3	74.3	88.0	71.6	25.1	0.44	0.40	0.28	
(x10 <sup>3</sup> )									
LL	1060	1116	1048	1020	193	0.41	0.83	0.52	
GSH-Rx									
PRAT	77.9	107.1	110.5	133.4	20	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.64	
Liver	5518	5214	5007	5098	631	0.16	0.63	0.37	
LL	121	102	116	117	23.6	0.53	0.29	0.23	
GSH - Liver	5389	6202	5318	5122	1433	0.68	0.91	0.34	
GSSG - Liver	275	286	283	257	162	0.83	0.88	0.77	
Ratio - Liver	23.7	29.2	26.4	21.9	14.8	0.66	0.93	0.35	
<b>Week 13-14 (n = 35)</b>									
SOD									
PRAT	42.4	53.9	53.4	82.5	18.9	0.004	<b>0.004</b>	0.18	
Liver	2594b	2392a	2507b	3257c	661	<b>0.09</b>	0.24	<b>0.05</b>	
LL	221	218	241	240	30	<b>0.05</b>	0.85	0.89	
CAT									
PRAT	781	810	812	1107	293	0.11	0.11	0.19	
Liver	834	961	1004	923	180	0.29	0.72	0.10	
(x10 <sup>3</sup> )									
LL	1006	960	852	945	200	0.23	0.73	0.31	
GSH-Rx									
PRAT	52.2	67.9	59.8	89.3	23	<b>0.07</b>	<b>0.007</b>	0.38	
Liver	4334	4783	5066	5391	672	<b>0.007</b>	0.11	0.78	
LL	98	100	103	105	18	0.42	0.83	0.92	

765 Pigs from two genetic lines divergently selected for low (LRFI) or high (HRFI) residual feed  
 766 intake (RFI) were housed either in good or poor hygiene conditions during the first 6 weeks  
 767 after their transfer in growing-finishing pens (period 1, challenge). Half of these pigs were  
 768 killed at week 6, whereas another half were placed in good hygiene conditions until slaughter  
 769 at weeks 13 or 14 (period 2, recovery). Activities of antioxidant enzymes such as glutathione  
 770 reductase (GSH-Rx), superoxide dismutase (SOD) and catalase (CAT) were assessed  
 771 (nM/min/g tissue) in perirenal adipose tissue (PRAT), liver and *longissimus lumborum* (LL)  
 772 muscle. Reduced (GSH) and oxidized (GSSG) forms of glutathione (expressed as pmol per  
 773 well) were also assessed in the liver. The ratio between reduced and oxidized forms  
 774 (GSH:GSSG) was calculated. Letters (a,b,c) were added in case of interaction (LxH;  $P <$   
 775 0.10) between hygiene (H) and RFI line (L), and means sharing a common letter did not  
 776 differ. MSE: root mean standard error of the statistical model. Bold face highlights significant



777 differences ( $P \leq 0.05$ ) between treatments, and when italicized, this denotes a trend ( $0.05 < P$   
778  $\leq 0.10$ ).  
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780 **Table 5 Effects of RFI line and hygiene conditions on oxidative enzymes in perirenal**  
 781 **adipose tissue of growing pigs**

	LRFI		HRFI		<i>P</i> values			
	Good	Poor	Good	Poor	MSE	Line	Hygiene	LxH
<b>Week 6 (n = 36)</b>								
HAD	4.42	5.55	4.42	5.60	1.59	0.96	<b>0.04</b>	0.96
CS	3.85	4.37	3.85	4.72	1.13	0.65	<b>0.07</b>	0.64
<b>Week 13-14 (n = 35)</b>								
HAD	3.78	4.12	3.56	4.53	1.4	0.84	0.17	0.51
CS	6.82	3.24	2.86	3.59	4.4	0.24	0.35	0.16

782 Pigs from two genetic lines divergently selected for low (LRFI) or high (HRFI) RFI were  
 783 housed either in good or poor hygiene conditions during the first 6 weeks after their transfer  
 784 in growing-finishing pens (period 1, challenge). Half of these pigs were killed at week 6,  
 785 whereas another half were placed in good hygiene conditions until slaughter at weeks 13 to 14  
 786 (period 2, recovery). Enzymes related to oxidative nutrient catabolism, namely beta-hydroxy-  
 787 acylCoA-dehydrogenase (HAD) and citrate synthase (CS) were measured ( $\mu\text{mole}/\text{min}/\text{g}$   
 788 tissue) in adipose tissue. LxH: interaction between hygiene (H) and RFI line (L). MSE: root  
 789 mean standard error of the statistical model. Bold face highlights significant differences ( $P \leq$   
 790 0.05) between treatments, and when italicized, this denotes a trend ( $0.05 < P \leq 0.10$ ).

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