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# Effects of Genetic Selection for Residual Feed Intake on Behavioral Reactivity of Castrated Male Pigs to Novel Stimuli Tests

## Abstract

Increasing feed efficiency in swine is important for increasing sustainable food production and profitability for producers; therefore, this is often selected for at breeding. Residual feed intake (RFI) can be used for the genetic selection of pigs for feed efficiency. In our selection project, low-RFI pigs consume less feed for equal weight gain compared to their less efficient, high-RFI counterparts. However, little is known about how feed efficiency influences the pig's behavioral reactivity toward fear-eliciting stimuli. In this study, behavioral reactivity of pigs divergently selected for RFI was evaluated using human approach- (HAT) and novel object tests (NOT). Forty low-RFI (more feed efficient) and 40 high-RFI (less feed efficient) castrated male pigs (barrows;  $46.5 \pm 8.6$  kg) from 8th generation Yorkshire RFI selection lines were randomly selected and evaluated once using HAT and once using NOT over a four week period utilizing a crossover experimental design. Each pig was individually tested within a  $4.9 \times 2.4$  m test arena for 10 min; behavior was evaluated using live and video observations. The test arena floor was divided into four zones; zone 1 being oral, nasal, and/or facial contact with the human (HAT) or orange traffic cone (NOT) and zone 4 being furthest from the human or cone and included the point where the pig entered the arena. During both HAT and NOT, low-RFI pigs crossed fewer zones ( $P < 0.0001$ ), had fewer head movements ( $P \leq 0.02$ ), defecated less frequently ( $P \leq 0.03$ ), displayed a shorter duration of freezing ( $P = 0.05$ ), and froze less frequently (HAT: low-RFI =  $4.9 \pm 0.65$  vs. high-RFI =  $7.5 \pm 0.96$ ; NOT: low-RFI =  $4.7 \pm 0.66$  vs. high-RFI =  $7.2 \pm 0.96$ ;  $P < 0.0001$ ) compared to high-RFI pigs. During HAT, low-RFI pigs also attempted to escape less frequently (low-RFI =  $0.4 \pm 0.14$  vs. high-RFI =  $1.1 \pm 0.30$ ;  $P = 0.001$ ) compared to high-RFI pigs. In contrast, compared to the high-RFI pigs, low-RFI pigs took 48 s longer during HAT and 52 s longer during NOT to approach zone 1 ( $P \leq 0.04$ ). These results indicate that low-RFI pigs had decreased behavioral reactivity during HAT and NOT compared to high-RFI pigs. This may suggest that reducing a pig's behavioral reactivity is an important component of improving feed efficiency; however, it may have implications for animal handling and facility design.

## Keywords

Swine Feed Efficiency, Pig, Feed efficiency, Stress, Fear, Human approach, Novel object

## Disciplines

Agriculture | Animal Sciences | Genetics and Genomics | Large or Food Animal and Equine Medicine

## Comments

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1       **Effects of genetic selection for residual feed intake on behavioral reactivity of**  
2                                       **castrated male pigs to novel stimuli tests**

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22 **ABSTRACT**

23 Increasing feed efficiency in swine is important for increasing sustainable food  
24 production and profitability for producers; therefore, this is often selected for at breeding.  
25 Residual feed intake (RFI) can be used for the genetic selection of pigs for feed  
26 efficiency. In our selection project, low-RFI pigs consume less feed for equal weight gain  
27 compared to their less efficient, high-RFI counterparts. However, little is known about  
28 how feed efficiency influences the pig's behavioral reactivity towards fear-eliciting stimuli.  
29 In this study, behavioral reactivity of pigs divergently selected for RFI was evaluated  
30 using human approach- (HAT) and novel object tests (NOT). Forty low-RFI (more feed  
31 efficient) and 40 high-RFI (less feed efficient) castrated male pigs (barrows;  $46.5 \pm 8.6$   
32 kg) from 8th generation Yorkshire RFI selection lines were randomly selected and  
33 evaluated once using HAT and once using NOT over a four week period utilizing a  
34 crossover experimental design. Each pig was individually tested within a 4.9 x 2.4 m test  
35 arena for 10 min; behavior was evaluated using live and video observations. The test  
36 arena floor was divided into four zones; zone 1 being oral, nasal, or facial contact with  
37 the human (HAT) or orange traffic cone (NOT) and zone 4 being furthest from the  
38 human or cone and included the point where the pig entered the arena. During both HAT  
39 and NOT, low-RFI pigs crossed fewer zones ( $P < 0.0001$ ), had fewer head movements  
40 ( $P \leq 0.02$ ), defecated less frequently ( $P \leq 0.03$ ), displayed a shorter duration of freezing  
41 ( $P = 0.05$ ), and froze less frequently (HAT: low-RFI =  $4.9 \pm 0.65$  vs. high-RFI =  $7.5 \pm$   
42  $0.96$ ; NOT: low-RFI =  $4.7 \pm 0.66$  vs. high-RFI =  $7.2 \pm 0.96$ ;  $P < 0.0001$ ) compared to  
43 high-RFI pigs. During HAT, low-RFI pigs also attempted to escape less frequently (low-  
44 RFI =  $0.4 \pm 0.14$  vs. high-RFI =  $1.1 \pm 0.30$ ;  $P = 0.001$ ) compared to high-RFI pigs. In  
45 contrast, compared to the high-RFI pigs, low-RFI pigs took 48 sec longer during HAT  
46 and 52 sec longer during NOT to approach zone 1 ( $P \leq 0.04$ ). These results indicate that  
47 low-RFI pigs had decreased behavioral reactivity during HAT and NOT compared to

48 high-RFI pigs. This may suggest that reducing a pig's behavioral reactivity is an  
49 important component of improving feed efficiency; however, **it** may have implications for  
50 animal handling and facility design.

51

52 *Keywords:* Pig, Feed efficiency, Stress, Fear, Human approach, Novel object

53

54 *Abbreviations:* RFI- Residual feed intake; HAT- Human approach test; NOT- Novel  
55 object test; HPA- hypothalamic-pituitary-adrenocortical

## 56 **1. Introduction**

57           Increasing feed efficiency is an important objective for livestock production.  
58 Better feed efficiency can improve producer profitability, increase production for feeding  
59 a growing world population and improve environmental sustainability (Nkrumah et al.,  
60 2006; Wall et al., 2010). In place of traditional gross efficiency (gain:feed) and feed  
61 conversion (feed:gain) ratios, many investigators have begun using residual feed intake  
62 (RFI) as an alternative method to measure feed efficiency (Koch et al., 1963; Cai et al.,  
63 2008). The Iowa State University Yorkshire RFI selection project uses a RFI model that  
64 defined the difference between the actual feed intake of an animal and its expected feed  
65 intake based on a given amount of growth and back fat. Therefore, pigs that consume  
66 less feed than expected for maintenance and growth have a lower RFI, are more feed  
67 efficient, and they are therefore economically better for lean production relative to higher  
68 RFI pigs (Young et al., 2011).

69           Numerous studies have been conducted to examine the physiology of feed  
70 efficiency using divergent RFI models. Specifically in pigs, RFI research has focused on  
71 feed intake patterns (Young et al., 2011), physical activity (Sadler et al., 2011), body  
72 composition (Boddicker et al., 2011a, b), nutrient digestibility (Barea et al., 2010; Harris  
73 et al., 2012), immune system activation (Rakhshandeh et al., 2012), skeletal muscle  
74 oxidative stress (Grubbs et al., 2013) and protein turnover (Cruzen et al., 2013).  
75 Furthermore, the hypothalamic-pituitary-adrenocortical (HPA) axis has been shown to be  
76 an important contributor to feed efficiency in pigs (Hennessy and Jackson, 1987), sheep  
77 (Knott et al., 2008, 2010), and poultry (Katie et al., 1988). These studies have revealed a  
78 relationship between higher feed efficiency and a lower glucocorticoid response.  
79 However, there is no consensus and it remains unclear whether improved feed efficiency  
80 alters behavior in livestock (Braastad and Katie, 1989; Luiting et al., 1994; Amdi et al.,  
81 2010).

82 Novelty has been utilized in studies of pig stress responses to a human  
83 approach- (HAT; Hemsworth et al., 1981; Gonyou et al., 1986; Janczak et al., 2003) and  
84 novel object test (NOT; Hemsworth et al., 1996; Dalmau et al., 2009; de Sevilla et al.,  
85 2009). An animal's response to HAT and NOT can help further our understanding of the  
86 animal's responsiveness to stress, which can in turn impact the animal's welfare during  
87 routine handling and husbandry. It was recently suggested that breeding for improved  
88 feed efficiency, and particularly for reduced RFI, may decrease the animal's ability to  
89 adapt to stress (Rydhmer and Canario, 2014). Therefore, the objective of this study was  
90 to examine the association between long-term divergent selection for RFI and behavioral  
91 reactivity to fear-eliciting stimuli. The hypothesis that low-RFI pigs would be less  
92 behaviorally reactive compared to high-RFI pigs was specifically tested by determining if  
93 divergent line selection for RFI influenced pigs' behavioral reactivity to HAT and NOT.  
94 These data will help develop breeding, handling, and management strategies to optimize  
95 feed efficiency in swine.

96

## 97 **2. Materials and methods**

98 All experimental procedures were approved by the Iowa State University Animal  
99 Care and Use Committee. This experiment was conducted over four consecutive weeks  
100 from October through November, 2011.

101

### 102 *2.1. Animals and housing*

103 A total of 80 healthy Yorkshire castrated male pigs (barrows;  $46.5 \pm 8.6$  kg test  
104 day body weight) divergently selected for RFI were used. Half (20 low-RFI and 20 high-  
105 RFI) of the pigs were fed a low-fiber, high-energy diet (9.4% neutral detergent fiber,  
106 13.86 MJ of metabolizable energy/kg of feed) and half were fed a high-fiber, low-energy  
107 diet (25.9% neutral detergent fiber, 11.97 MJ of metabolizable energy/kg of feed). Both

108 diets met or exceeded NRC (1998) requirements and further information regarding  
109 ingredient and nutrient analysis is explained by Colpoys and colleagues (2014). Two  
110 genetic line treatments were compared: low-RFI (n=40) and high-RFI (n=40). Divergent  
111 line selection criteria were based on estimate breeding values for RFI as explained by  
112 Cai and colleagues (2008). The low-RFI genetic line had been selected over eight  
113 generations whereas the high-RFI genetic line had been randomly selected over five  
114 generations, and then selectively bred for high-RFI over the next three generations.

115         This work was conducted at the Lauren Christian Swine Research Center at the  
116 Iowa State University Bilsland Memorial Farm, near Madrid, Iowa, USA. All pigs were  
117 housed in a conventional confinement unit within one room containing 12 mixed-sex and  
118 mixed-line pens of 15 to 16 pigs/pen; five to eight pigs from each pen were tested. The  
119 pigs were moved to this facility 10 days prior to the start of the experiment. Each pen  
120 measured 5.6 m long x 2.3 m wide and had a slatted concrete floor. The barn was  
121 naturally ventilated with side curtains. Each pen contained an electronic one-space  
122 feeder (FIRE<sup>®</sup>, Osborne Industries, Inc., Osborne, KS, USA) that recorded the feed  
123 intake of each pig, positioned at the front of the pen to provide pigs with *ad libitum* feed.  
124 Water was provided *ad libitum* through two nipple-type waterers (Edstrom, Waterford,  
125 WI, USA) per pen. One electronic recording device (HOBO Pro v2, temp / RH, U23-001,  
126 Onset Computer Corporation, Bourne, MA, USA) located in the center of the room, 2.2  
127 m from the ground, recorded ambient temperature (°C) and relative humidity (%) every 5  
128 min for the duration of the trial. The mean ( $\pm$ S.D.) ambient temperature was 21.7  
129 ( $\pm$ 1.9)°C and relative humidity was 70.5 ( $\pm$ 9.8)%.

130

## 131 *2.2. Test methodology and facility*

132         Pig testing occurred 5 days per week over four consecutive weeks. A testing  
133 session consisted of a 10 min period during which the individual pig underwent HAT or



134 NOT within the experimental arena. All tests were performed between 13:00 and 17:00  
135 h. A total of 40 pigs (20 low-RFI and 20 high-RFI) were randomly selected using a  
136 random number generator (Microsoft Excel 2010, Microsoft Corporation, Santa Rosa,  
137 CA, USA) to be tested using HAT first and the remaining 40 pigs (20 low-RFI and 20  
138 high-RFI) experienced NOT first. Pigs then experienced the opposite test 1 week later,  
139 utilizing a crossover experimental design. Therefore, each pig was tested a total of two  
140 times, once using each test. Genetic line and diet were blocked by time so that within  
141 each hour each of the following types were tested in random order: low-RFI high-fiber  
142 diet, low-RFI low-fiber diet, high-RFI high-fiber diet, and high-RFI low-fiber diet. Pigs  
143 were tested in the same order for both tests and at the same time of day, and the  
144 individual pig was the experimental unit.

145         The HAT and NOT were conducted in a rectangular arena separate from the  
146 home pens. The arena setup was adapted from published work by Hemsworth and  
147 colleagues (1989) and Marchant-Forde and others (2003). The arena measured 4.9 m  
148 long x 2.4 m wide and had 1.2 m high, black corrugated plastic sides that were attached  
149 to gates. In order to hide the human observer visually during NOT, a 1.2 m wide x 2.2 m  
150 high black corrugated plastic observation hide was positioned outside the arena.  
151 Concentric curves were drawn on the slatted concrete floor using permanent marker one  
152 day before the start of testing to divide the arena into four zones in order to measure the  
153 location of the pig in proximity to the novel stimulus. Zone 1 was defined as oral, nasal,  
154 and/or facial contact with the human or the cone during HAT and NOT, respectively. For  
155 consistency with the other zones, pigs that touched the human or the cone will be  
156 referred to as entering zone 1. Zone 2 was the area nearest to the novel stimulus and  
157 zone 4 was the area where the pig entered the test arena, furthest from the novel  
158 stimulus. Zones 2, 3, and 4 consisted of approximately equal area which allowed the  
159 entire body of the pig to fit within the zone. The concentric curves allowed each zone to

160 measure a consistent distance from the novel stimulus (Fig. 1). Located in the center of  
161 the arena, 2.3 m from the ground, was one electronic recording device (HOBO Pro v2,  
162 temp / RH, U23-001, Onset Computer Corporation, Bourne, MA, USA) that recorded  
163 ambient temperature ( $^{\circ}\text{C}$ ) and relative humidity (%) every 5 min for the duration of the  
164 testing. Throughout the testing period, the mean ( $\pm$ S.D.) ambient temperature was 15.3  
165 ( $\pm$ 2.2) $^{\circ}\text{C}$ , 6.4 $^{\circ}\text{C}$  cooler than the home pen, and relative humidity was 73.2 ( $\pm$ 10.6)%,  
166 2.7% higher than the home pen.

167 Three color cameras (Panasonic, Model WV-CP-484, Matsushita Co. LTD.,  
168 Kadoma, Japan) were positioned 2.1 m above the test arena. Camera 1 was positioned  
169 over zone 1, camera 2 captured zones 2 and 3 and camera 3 captured zone 4. The  
170 cameras were fed into a multiplexer using Noldus Portable Lab (Noldus Information  
171 Technology, Wageningen, The Netherlands) and time-lapse video was collected onto a  
172 computer using HandyAVI (HandyAVI version 4.3 D, Anderson's AZcendant Software,  
173 Tempe, AZ, USA) at 10 frames/s.

174 One handler removed the pig to be tested from its home pen using a sort board.  
175 Each pig was moved down an alleyway (0.30 m to 12.47 m long x 0.79 m wide) into a  
176 weigh scale (1.50 m long x 0.5 m wide; Electronic Weighing Systems, Rite Weigh,  
177 Robert E Spencer Enterprises, Ackley, IA, USA) adjacent to the test arena. The pig  
178 remained in the weigh scale for one min while the pig's weight was collected to create a  
179 uniform pre-test environment for every pig. Black corrugated plastic was attached to the  
180 front of the weigh scale so the pigs were not able to see into the test arena. At the  
181 conclusion of the min, the weigh scale door was opened and the pig was allowed to  
182 enter zone 4 of the arena. If the pig did not enter the arena within 15 s of the scale door  
183 opening, the handler gently pushed the pig forward using their hands. The test time  
184 began when both front hooves entered zone 4. Following the 10 min testing period, each  
185 pig was returned to its home pen by the handler using the same methods as previously

186 described. Feces and urine within the test arena were scraped through the slats  
187 following each testing session and the test arena was hosed down with water at the end  
188 of each testing day.

189

#### 190 *2.2.1. Human approach test*

191 Each pig was individually assessed using HAT, which was designed to measure  
192 responses to an unfamiliar human stimulus. The human stimulus was the same woman  
193 for all tests, and had never previously interacted with the pigs. The human showered into  
194 the facility using the same products at the start of each testing day. During testing, the  
195 unfamiliar human wore orange coveralls and orange boots, stood silently at the center of  
196 the opposite wall (zone 1) holding a clipboard, and did not interact with or move toward  
197 the pigs. Minimal arm movement and body shifting occurred during live observation and  
198 data collection. At the end of each testing day, coveralls were laundered and boots were  
199 hosed off with water.

200

#### 201 *2.2.2. Novel object test*

202 Each pig was individually assessed using NOT, which was designed to measure  
203 responses to an unfamiliar object stimulus, an orange traffic cone. The traffic cone was  
204 positioned at the center of the opposite wall (zone 1) and was hosed off with water at the  
205 end of each testing day. The same woman from the HAT collected live observations  
206 from outside the test arena behind the black corrugated plastic observation hide (Fig. 1).

207

#### 208 *2.3. Measures*

209 Live observations for the frequency of eliminatory behaviors were continuously  
210 collected during both tests (Dawkins et al., 2007). Video observations were continuously  
211 recorded (Dawkins et al., 2007) using the Observer software (The Observer XT version

212 10.5, Noldus Information Technology, Wageningen, The Netherlands) to decode  
213 approach, head orientation, freezing, and escape attempts (Table 1). All live and video  
214 observations were collected by the same, trained researcher who was blind to genetic  
215 line and diet treatments.

216

## 217 *2.4. Data analysis*

218 All data were evaluated for normality using the Shapiro-Wilk test and Q-Q plots  
219 using SAS (SAS version 9.3, SAS Inst. Inc., Cary, NC). Data were not normally  
220 distributed; therefore, data were analyzed using the Glimmix procedure of SAS. All HAT  
221 and NOT data were analyzed separately. Pigs fed high-fiber diets were found to  
222 defecate more frequently during HAT, attempt to escape more frequently during NOT,  
223 and crossed fewer zone lines during NOT compared to pigs fed low-fiber diets. However,  
224 the main effects of diet have been presented previously (Colpoys et al., 2014) and are  
225 therefore not presented in the current work. Latency data were analyzed with a gamma  
226 distribution, duration data were analyzed with a beta distribution, and frequency data  
227 were analyzed with a poisson distribution. During both tests, one low-RFI pig did not  
228 enter zone 1; therefore, was given a latency of 600 s. All behaviors were analyzed using  
229 a model with the fixed effects of genetic line and test week, covariate of body weight  
230 (measured prior to each test), and random effect of pen nested within diet. The  
231 significance level was fixed at  $P \leq 0.05$ .

232

## 233 **3. Results**

### 234 *3.1. Human approach test*

#### 235 *3.1.1. Stimulus attention*

236 Low-RFI pigs took 48 s longer to enter zone 1 compared to the high-RFI pigs ( $P$   
237 = 0.04). No differences were observed between lines for total number of zone 1

238 entrances ( $P = 0.16$ ) or duration of time spent within zone 1 ( $P = 0.93$ ; Table 2). Duration  
239 of time spent within zone 2 ( $F_{1,63} = 0.8$ ,  $P = 0.37$ ), 3 ( $F_{1,63} = 0.01$ ,  $P = 0.93$ ), and 4 ( $F_{1,63} =$   
240  $0.04$ ,  $P = 0.85$ ) did not differ between lines. Furthermore, no line differences were  
241 observed in duration of time spent with head in front ( $F_{1,63} = 0.2$ ,  $P = 0.63$ ), side ( $F_{1,63} =$   
242  $2.4$ ,  $P = 0.13$ ), or back ( $F_{1,63} = 0.7$ ,  $P = 0.40$ ) orientation relative to the human.

243

### 244 *3.1.2. Arousal and fear behavior*

245 Compared to high-RFI pigs, low-RFI pigs crossed fewer zone lines ( $P < 0.0001$ )  
246 and had fewer head movements ( $P = 0.02$ ). No difference was observed between lines  
247 for frequency of urinations ( $P = 0.43$ ); however, low-RFI pigs defecated fewer times  
248 compared to high-RFI pigs ( $P = 0.002$ ). Low-RFI pigs performed escape attempts less  
249 frequently compared to high-RFI pigs ( $P = 0.001$ ); however, no difference was observed  
250 between lines in duration of time spent attempting to escape ( $P = 0.08$ ). Compared to  
251 high-RFI pigs, low-RFI pigs froze less often ( $P < 0.0001$ ) and spent 2% less time  
252 freezing ( $P = 0.05$ ; Table 2).

253

### 254 *3.2. Novel object test*

#### 255 *3.2.1. Stimulus attention*

256 Low-RFI pigs took 52 s longer to first enter zone 1 compared to the high-RFI pigs  
257 ( $P = 0.02$ ). No differences were observed between lines for total number of zone 1  
258 entrances ( $P = 0.13$ ) or duration of time spent within zone 1 ( $P = 0.93$ ; Table 3). Duration  
259 of time spent within zone 2 ( $F_{1,63} = 1.1$ ,  $P = 0.31$ ), 3 ( $F_{1,63} = 1.3$ ,  $P = 0.25$ ), and 4 ( $F_{1,63} =$   
260  $0.00$ ,  $P = 0.99$ ) did not differ between lines. Furthermore, no line differences were  
261 observed in duration of time spent with head in front ( $F_{1,63} = 0.6$ ,  $P = 0.44$ ), side ( $F_{1,63} =$   
262  $0.2$ ,  $P = 0.68$ ), or back ( $F_{1,63} = 0.9$ ,  $P = 0.34$ ) orientation relative to the cone.

263

264 *3.2.2. Arousal and fear behavior*

265 Compared to high-RFI pigs, low-RFI pigs crossed fewer zone lines ( $P < 0.0001$ )  
266 and had fewer head movements ( $P = 0.01$ ). No difference was observed between lines  
267 for frequency of urinations ( $P = 0.60$ ); however, low-RFI pigs defecated fewer times  
268 compared to high-RFI pigs ( $P = 0.03$ ). No difference was observed between lines in total  
269 number of escape attempts ( $P = 0.21$ ) or duration of time spent attempting to escape ( $P$   
270  $= 0.35$ ). Compared to high-RFI pigs, low-RFI pigs froze less often ( $P < 0.0001$ ) and  
271 spent 2% less time freezing ( $P = 0.05$ ; Table 3).

272

273 **4. Discussion**

274 *4.1. Stimulus attention*

275 Low-RFI pigs took longer to approach zone 1 compared to high-RFI pigs. These  
276 results are similar to those of Hayne and Gonyou (2006), who reported that pigs with a  
277 higher average daily gain were slower to approach a human. In the current study,  
278 latency to approach zone 1 was a primary outcome, on the assumption that fearful  
279 animals would be less likely to approach. However, this assumption does not coincide  
280 with other measures of stimuli attention, as the frequency of entrances, the duration of  
281 time spent within and oriented towards zone 1 did not differ between lines. Furthermore,  
282 this assumption seems to be inconsistent with the arousal and fear behaviors in the  
283 current experiment. Two possible, non-mutually exclusive explanations of latency to  
284 approach zone 1 will be discussed.

285 One explanation is that the high-RFI pigs were more fearful of social isolation  
286 than the human or cone. Social isolation can be distressing for pigs (Gonyou, 2001) and  
287 is likely one of the greatest stressors during HAT and NOT (Forkman et al., 2007; Pairis  
288 et al., 2009). A second explanation is that approach latency reflects coping style rather  
289 than the level of fear. Hayne and Gonyou (2006) proposed that a fast approach is

290 indicative of an active response whereas a slow approach is indicative of a passive  
291 response in pigs. This interpretation is further supported by Hessing and colleagues  
292 (1994), who reported that pigs quicker to approach a novel object were also more  
293 resistant to a back test. These two explanations should be considered in future research.

294

#### 295 *4.2. Arousal and fear behavior*

296 Low-RFI pigs were less active compared to high-RFI pigs, as indicated by less  
297 frequent zone crossings and head orientation changes. Reduced activity in the low-RFI  
298 line was similar to home pen behavior in fifth generation gilts from the same selection  
299 line project (Sadler et al., 2011). Likewise, Imrich and colleagues (2012) reported that  
300 pigs with increased average daily gain were less active during a habituation (novel  
301 arena) test. The opposite relationship has been found in sheep selected for behavioral  
302 activity during fearful situations, where less active sheep were less feed efficient than  
303 more active sheep (Amdi et al., 2010).

304 During HAT, low-RFI pigs attempted to escape fewer times compared to high-  
305 RFI pigs. During both tests, low-RFI pigs defecated less often and engaged in fewer  
306 freezing postures. This relationship between improved performance traits and reduced  
307 fearfulness is consistent with work by Geverink and colleagues (2004), who reported that  
308 gilts with fewer escape attempts during a back test had higher average daily gain and  
309 metabolizable energy.

310

#### 311 *4.3. General discussion*

312 Preliminary analysis of the HPA axis in these lines of pigs reported that low-RFI  
313 gilts tended to have lower cortisol concentrations both before and after an exogenous  
314 adrenocorticotropin hormone challenge compared to high-RFI gilts (Jenkins et al., 2013).  
315 Therefore, the reduced behavioral reactivity seen in low-RFI pigs compared to high-RFI

316 pigs may in part be due to a reduced physiological stress response. Furthermore, eighth  
317 generation, high-RFI pigs from first parity sows (in contrast to pigs from second parity  
318 sows investigated in the current study) had 241 g/d greater RFI and 2.5 mm greater  
319 backfat than low-RFI pigs (Young and Dekkers, 2012). Reduced feed efficiency and  
320 increased carcass fat has been observed in stressed pigs within the commercial  
321 environment (Black et al., 2001); therefore, may suggest greater stress in high-RFI than  
322 low-RFI pigs within the commercial home pen environment.

323         Improving swine feed efficiency is important for producer profitability,  
324 sustainability, and resource allocation. Therefore, improving feed efficiency has become  
325 a goal of genetic improvement and management practices in livestock species.  
326 However, when selectively breeding pigs for feed efficiency, it is important to take the  
327 animal's welfare into consideration. One aspect of the animal's welfare which may be  
328 influenced by selective breeding is the animal's stress response, particularly to human  
329 interaction and novel stimuli. Our data presented herein, indicate that low-RFI pigs  
330 (increased feed efficiency) had decreased behavioral reactivity during HAT and NOT  
331 compared to high-RFI pigs.

332

## 333 **5. Conclusions**

334         Compared to selection for reduced feed efficiency (high-RFI), selective breeding  
335 for increased feed efficiency (low-RFI) appears to have resulted in an animal welfare  
336 benefit in terms of calmer pigs that are less reactive to novelty. Nevertheless, the more  
337 feed efficient pigs took longer to approach the novel stimuli compared to the less feed  
338 efficient pigs, which may have implications for animal handling and facility design.  
339 Furthermore, these results may suggest that reducing an animal's stress response is an  
340 important component of conserving energy for growth and improving feed efficiency.



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348

349 **References**

- 350 Amdi, C., Williams, A.R., Maloney, S.K., Tauson, A.H., Knott, S.A., Blache, D., 2010.  
351 Relationship between behavioural reactivity and feed efficiency in housed sheep.  
352 Animal Production Science 50, 683-687.
- 353 Barea, R., Dubois, S., Gilbert, H., Sellier, P., van Milgen, J., Noblet, J., 2010. Energy  
354 utilization in pigs selected for high and low residual feed intake. Journal of Animal  
355 Science 88, 2062-2072.
- 356 Black, J.L., Giles, L.R., Wynn, P.C., Knowles, A.G., Kerr, C.A., Jones, M.R., Strom, A.D.,  
357 Gallagher, N.L., Eamens, G.J., 2001. A review - Factors limiting the performance  
358 of growing pigs in commercial environments, 8th Biennial Conference of the  
359 Australasian Pig Science Association, Adelaide, Australia, pp. 9-36.
- 360 Boddicker, N., Gabler, N.K., Spurlock, M.E., Nettleton, D., Dekkers, J.C.M., 2011a.  
361 Effects of ad libitum and restricted feed intake on growth performance and body  
362 composition of Yorkshire pigs selected for reduced residual feed intake. Journal  
363 of Animal Science 89, 40-51.
- 364 Boddicker, N., Gabler, N.K., Spurlock, M.E., Nettleton, D., Dekkers, J.C.M., 2011b.  
365 Effects of ad libitum and restricted feeding on early production performance and

366 body composition of Yorkshire pigs selected for reduced residual feed intake.  
367 Animal 5, 1344-1353.

368 Braastad, B.O., Katle, J., 1989. Behavioural differences between laying hen populations  
369 selected for high and low efficiency of food utilisation. British Poultry Science 30,  
370 533-544.

371 Cai, W., Casey, D.S., Dekkers, J.C.M., 2008. Selection response and genetic  
372 parameters for residual feed intake in Yorkshire swine. Journal of Animal Science  
373 86, 287-298.

374 Colpoys, J.D., Johnson, A.K., Gabler, N.K., Keating, A.F., Millman, S.T., Siegford, J.M.,  
375 2014. Pig behavioral responsiveness to a human or novel object when fed low  
376 versus high energy diets, Iowa State University Animal Industry Report AS 660,  
377 ASL R2915. Available at: [http://lib.dr.iastate.edu/ans\\_air/vol660/iss1/80](http://lib.dr.iastate.edu/ans_air/vol660/iss1/80)

378 Cruzen, S.M., Harris, A.J., Hollinger, K., Punt, R.M., Grubbs, J.K., Selsby, J.T., Dekkers,  
379 J.C.M., Gabler, N.K., Lonergan, S.M., Huff-Lonergan, E., 2013. Evidence of  
380 decreased muscle protein turnover in gilts selected for low residual feed intake.  
381 Journal of Animal Science 91, 4007-4016.

382 Dalmau, A., Fabrega, E., Velarde, A., 2009. Fear assessment in pigs exposed to a novel  
383 object test. Applied Animal Behaviour Science 117, 173-180.

384 Dawkins, M.S., Zoologiste, G.-B., Zoologist, G.B., 2007. Observing animal behaviour:  
385 design and analysis of quantitative data. Oxford University Press Oxford & New  
386 York.

387 de Sevilla, X.F., Casellas, J., Tibau, J., Fabrega, E., 2009. Consistency and influence on  
388 performance of behavioural differences in Large White and Landrace purebred  
389 pigs. Applied Animal Behaviour Science 117, 13-19.

390 Forkman, B., Boissy, A., Meunier-Salauen, M.C., Canali, E., Jones, R.B., 2007. A critical  
391 review of fear tests used on cattle, pigs, sheep, poultry and horses. *Physiology &*  
392 *Behavior* 92, 340-374.

393 Geverink, N.A., Heetkamp, M.J.W., Schouten, W.G.P., Wiegant, V.M., Schrama, J.W.,  
394 2004. Backtest type and housing condition of pigs influence energy metabolism.  
395 *Journal of Animal Science* 82, 1227-1233.

396 Gonyou, H.W., 2001. The social behaviour of pigs. *Social Behaviour in Farm Animals*.  
397 Keeling, LJ & Gonyou, HW (Eds.). CAB International, Wallingford, UK, 147-176.

398 Gonyou, H.W., Hemsworth, P.H., Barnett, J.L., 1986. Effects of frequent interactions with  
399 humans on growing pigs. *Applied Animal Behaviour Science* 16, 269-278.

400 Grubbs, J.K., Fritchen, A.N., Huff-Lonergan, E., Dekkers, J.C.M., Gabler, N.K.,  
401 Lonergan, S.M., 2013. Divergent genetic selection for residual feed intake  
402 impacts mitochondria reactive oxygen species production in pigs. *Journal of*  
403 *Animal Science* 91, 2133-2140.

404 Harris, A.J., Patience, J.F., Lonergan, S.M., Dekkers, J.C.M., Gabler, N.K., 2012.  
405 Improved nutrient digestibility and retention partially explains feed efficiency  
406 gains in pigs selected for low residual feed intake. *Journal of Animal Science* 90,  
407 164-166.

408 Hayne, S.M., Gonyou, H.W., 2006. Behavioural uniformity or diversity? Effects on  
409 behaviour and performance following regrouping in pigs. *Applied Animal*  
410 *Behaviour Science* 98, 28-44.

411 Hemsworth, P.H., Barnett, J.L., 1992. The effects of early contact with humans on the  
412 subsequent level of fear of humans in pigs. *Applied Animal Behaviour Science*  
413 35, 83-90.

414 Hemsworth, P.H., Barnett, J.L., Coleman, G.J., Hansen, C., 1989. A study of the  
415 relationships between the attitudinal and behavioural profiles of stockpersons

416 and the level of fear of humans and reproductive performance of commercial  
417 pigs. *Applied Animal Behaviour Science* 23, 301-314.

418 Hemsworth, P.H., Barnett, J.L., Hansen, C., 1981. The influence of handling by humans  
419 on the behavior, growth, and corticosteroids in the juvenile female pig. *Hormones*  
420 *and Behavior* 15, 396-403.

421 Hemsworth, P.H., Price, E.O., Borgwardt, R., 1996. Behavioural responses of domestic  
422 pigs and cattle to humans and novel stimuli. *Applied Animal Behaviour Science*  
423 50, 43-56.

424 Hennessy, D., Jackson, P., 1987. Relationship between adrenal responsiveness and  
425 growth rate, Manipulating pig production. I. Proceedings of the inaugural  
426 conference of the Australasian Pig Science Association (APSA), Albury,  
427 Australia, p. 23.

428 Helsing, M.J.C., Hagelsø, A.M., Schouten, W.G.P., Wiepkema, P.R., Van Beek, J.A.M.,  
429 1994. Individual behavioral and physiological strategies in pigs. *Physiology &*  
430 *Behavior* 55, 39-46.

431 Imrich, I., Mlyneková, E., Mlynek, J., Mad'arová, L., Kanka, T., Moravcová, L., 2012.  
432 Behavioural activity in pigs at habitual test in relation to production traits. *Polish*  
433 *Journal of Natural Sciences* 27, 257-267.

434 Janczak, A.M., Pedersen, L.J., Bakken, M., 2003. Aggression, fearfulness and coping  
435 styles in female pigs. *Applied Animal Behaviour Science* 81, 13-28.

436 Jenkins, J.D., Gabler, N.K., Anderson, L.L., Dekkers, J.C.M., Johnson, A.K., Dunshea,  
437 F.R., 2013. Evaluation of the responsiveness of swine divergently selected for  
438 feed efficiency to an exogenous adrenocorticotropin hormone (ACTH) challenge,  
439 Iowa State University Animal Industry Report AS 659, ASL R2814. Available at:  
440 [http://lib.dr.iastate.edu/ans\\_air/vol659/iss1/65](http://lib.dr.iastate.edu/ans_air/vol659/iss1/65)

441 Katle, J., Hamet, N., Durand, L., Rombauts, P., Merat, P., 1988. Divergent lines for  
442 residual feed intake of layers: response of chicks to inoculation by *Eimeria*  
443 *acervulina* and comparison of biological parameters. *Genetics Selection*  
444 *Evolution* 20, 387-396.

445 Knott, S.A., Cummins, L.J., Dunshea, F.R., Leury, B.J., 2008. Rams with poor feed  
446 efficiency are highly responsive to an exogenous adrenocorticotropin hormone  
447 (ACTH) challenge. *Domestic Animal Endocrinology* 34, 261-268.

448 Knott, S.A., Cummins, L.J., Dunshea, F.R., Leury, B.J., 2010. Feed efficiency and body  
449 composition are related to cortisol response to adrenocorticotropin hormone and  
450 insulin-induced hypoglycemia in rams. *Domestic Animal Endocrinology* 39, 137-  
451 146.

452 Koch, R.M., Swiger, L.A., Chambers, D., Gregory, K.E., 1963. Efficiency of feed use in  
453 beef cattle. *Journal of Animal Science* 22, 486-494.

454 Luiting, P., Urff, E.M., Verstegen, M.W.A., 1994. Between-animal variation in biological  
455 efficiency as related to residual feed consumption. *Netherlands Journal of*  
456 *Agricultural Science* 42, 59-67.

457 Marchant-Forde, J.N., Bradshaw, R.H., Marchant-Forde, R.M., Broom, D.M., 2003. A  
458 note on the effect of gestation housing environment on approach test measures  
459 in gilts. *Applied Animal Behaviour Science* 80, 287-296.

460 Nkrumah, J.D., Okine, E.K., Mathison, G.W., Schmid, K., Li, C., Basarab, J.A., Price,  
461 M.A., Wang, Z., Moore, S.S., 2006. Relationships of feedlot feed efficiency,  
462 performance, and feeding behavior with metabolic rate, methane production, and  
463 energy partitioning in beef cattle. *Journal of Animal Science* 84, 145-153.

464 Nutrition, N.R.C.S.o.S., 1998. Nutrient requirements of swine. National Academies  
465 Press.

466 Pairis, M., Young, A., Millman, S., Garvey, J., Johnson, A., 2009. Can fear be effectively  
467 assessed in Swine? A study measuring fear levels during a human approach  
468 test, Iowa State University Animal Industry Report AS 655, ASL R2470. Available  
469 at: [http://lib.dr.iastate.edu/ans\\_air/vol655/iss1/92](http://lib.dr.iastate.edu/ans_air/vol655/iss1/92)

470 Rakhshandeh, A., Dekkers, J.C.M., Kerr, B.J., Weber, T.E., English, J., Gabler, N.K.,  
471 2012. Effect of immune system stimulation and divergent selection for residual  
472 feed intake on digestive capacity of the small intestine in growing pigs. *Journal of*  
473 *Animal Science* 90, 233-235.

474 Rydhmer, L., Canario, L., 2014. Behavioral genetics in pigs and relations to welfare, in:  
475 Grandin, T., Deesing, M.J. (Eds.), *Genetics and the behavior of domestic*  
476 *animals*, Academic Press, San Diego, pp. 397-434.

477 Sadler, L.J., Johnson, A.K., Lonergan, S.M., Nettleton, D., Dekkers, J.C.M., 2011. The  
478 effect of selection for residual feed intake on general behavioral activity and the  
479 occurrence of lesions in Yorkshire gilts. *Journal of Animal Science* 89, 258-266.

480 Wall, E., Simm, G., Moran, D., 2010. Developing breeding schemes to assist mitigation  
481 of greenhouse gas emissions. *Animal* 4, 366-376.

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

485 Young, J.M., Dekkers, J.C.M., 2012. The genetic and biological basis of residual feed  
486 intake as a measure of feed efficiency, in: Patience, J.F. (Ed.), *Feed efficiency in*  
487 *swine*, Wageningen Academic Publishers, Wageningen, the Netherlands, pp.  
488 153-166.

489 **Table 1.** Ethogram of behaviors recorded during human approach and novel object  
 490 tests. Latency in seconds (s), duration (%), and/or frequency (n) of behaviors collected.  
 491 Ethogram adapted from Dalmau et al. (2009) and Hemsworth and Barnett (1992). Live  
 492 observations were utilized to collect elimination and video decoding was utilized to  
 493 collect all other measures.

<b>Measure</b>	<b>Description</b>
<b>Approach</b>	
Zone 1 (s, n, %)	The mouth, nose, and/or face of the pig contact any part of zone 1 (defined as the human or traffic cone). Latency was measured from the start of the test to the first zone 1 entrance
Zone 4, 3, & 2 (%)	The base of both the pig's ears were within the limits of the respective zone and the pig's mouth, nose, and/or face was not touching zone 1
Zone crossings (n)	Sum of the total number of zone 4, 3, and 2 entrances
<b>Head orientation</b>	
Front, Side, Back (%)	The pig's snout was pointed towards, perpendicular, or in the opposite direction of zone 1, respectively
Head movements (n)	The sum of front, side, and back head orientations
<b>Elimination</b>	
Urination (n)	Excreting urine
Defecation (n)	Excreting feces
<b>Escape attempt</b> (n, %)	The front two or all four pig's hooves were off the arena floor in attempt to remove itself from the test arena. Duration was measured from the removal of the two front hooves from the floor to all four hooves returning to the floor

**Freezing** (n, %)

No movement of any portion of the pig's body was visible for  $\geq 3$  s.  
Duration was measured from the start of the freeze to any  
movement of the body

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494



495 **Table 2.** Latency (s), frequency (n), and duration (%; least square means  $\pm$  SE) of  
 496 behaviors during the human approach test in castrated male pigs selected for low-RFI  
 497 (more feed efficient) and high-RFI (less feed efficient).

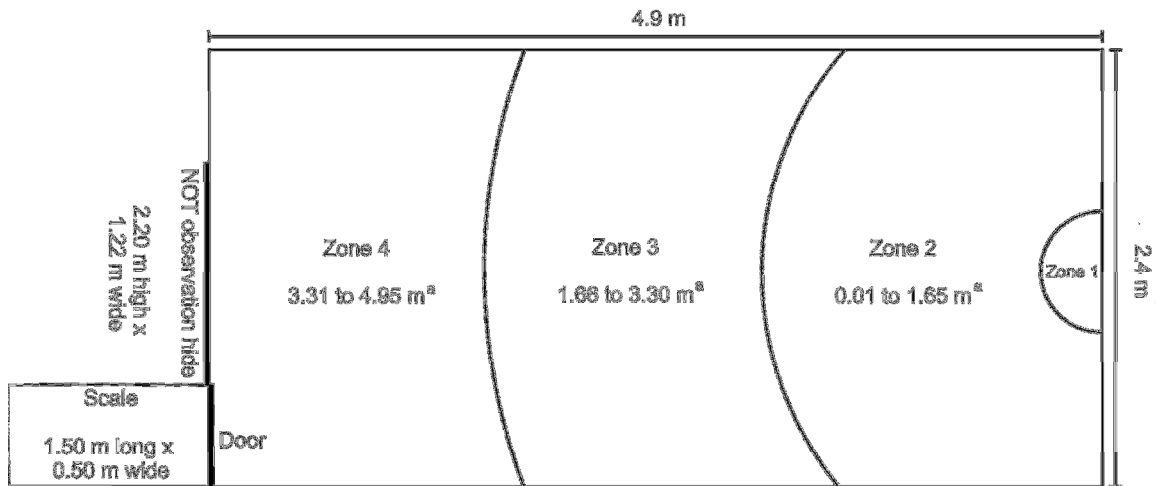
Measures	Genetic line		$F_{1,63}$	P-value
	Low-RFI (n = 40)	High-RFI (n = 40)		
Zone 1, s	132.7 $\pm$ 19.67	84.5 $\pm$ 12.47	4.5	0.04
Zone 1, n	6.5 $\pm$ 0.55	7.4 $\pm$ 0.60	2.1	0.16
Zone 1, %	10.6 $\pm$ 1.49	10.4 $\pm$ 1.47	0.01	0.93
Zone crossings, n	40.6 $\pm$ 1.71	48.9 $\pm$ 1.99	26.6	<0.0001
Head movements, n	86.7 $\pm$ 1.80	92.2 $\pm$ 1.87	6.0	0.02
Urination, n	0.5 $\pm$ 0.11	0.5 $\pm$ 0.12	0.1	0.72
Defecation, n	3.4 $\pm$ 0.30	4.9 $\pm$ 0.36	10.5	0.002
Escape attempt, n	0.4 $\pm$ 0.14	1.1 $\pm$ 0.30	11.3	0.001
Escape attempt, %	0.1 $\pm$ 0.04	0.2 $\pm$ 0.07	3.3	0.08
Freeze, n	4.9 $\pm$ 0.65	7.5 $\pm$ 0.96	22.8	<0.0001
Freeze, %	4.1 $\pm$ 0.71	6.2 $\pm$ 0.93	4.1	0.05

498

499 **Table 3.** Latency (s), frequency (n), and duration (%; least square means  $\pm$  SE) of  
 500 behaviors during the novel object test in castrated male pigs selected for low-RFI (more  
 501 feed efficient) and high-RFI (less feed efficient).

<b>Measures</b>	<b>Genetic line</b>		<b><i>F</i><sub>1,63</sub></b>	<b><i>P</i>-value</b>
	<b>Low-RFI (n = 40)</b>	<b>High-RFI (n = 40)</b>		
Zone 1, s	128.1 $\pm$ 20.84	76.3 $\pm$ 12.31	5.3	0.02
Zone 1, n	7.3 $\pm$ 0.63	8.3 $\pm$ 0.70	2.4	0.13
Zone 1, %	9.2 $\pm$ 1.46	9.1 $\pm$ 1.42	0.01	0.93
Zone crossings, n	40.1 $\pm$ 1.74	48.4 $\pm$ 2.03	28.5	<0.0001
Head movements, n	80.5 $\pm$ 2.28	86.2 $\pm$ 2.39	7.2	0.01
Urination, n	0.6 $\pm$ 0.12	0.5 $\pm$ 0.11	0.3	0.60
Defecation, n	3.4 $\pm$ 0.30	4.4 $\pm$ 0.35	4.7	0.03
Escape attempt, n	0.6 $\pm$ 0.18	0.9 $\pm$ 0.23	1.6	0.21
Escape attempt, %	0.1 $\pm$ 0.05	0.2 $\pm$ 0.07	0.9	0.35
Freeze, n	4.7 $\pm$ 0.66	7.2 $\pm$ 0.96	20.5	<0.0001
Freeze, %	3.9 $\pm$ 0.72	6.0 $\pm$ 0.92	3.9	0.05

502



503  
 504 **Figure 1.** Arena where pigs were tested using human approach- (HAT) and novel object  
 505 tests (NOT).

506 <sup>a</sup>Indicates the distance of each zone from the human or cone, located in zone 1. Zones  
 507 2, 3, and 4 consisted of approximately equal area.