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

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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 towards 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 ±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 x 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, 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 ± 0.65 vs. high-RFI = 7.5 ± 0.96; NOT: low-RFI = 4.7 ± 0.66 vs. high-RFI = 7.2 ± 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 ± 0.14 vs. high-RFI = 1.1 ± 0.30; $P = 0.001$) compared to high-RFI pigs. In contrast, compared to the high-RFI pigs, low-RFI pigs took 48 sec longer during HAT and 52 sec 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: Pig, Feed efficiency, Stress, Fear, Human approach, Novel object

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

1. Introduction

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

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

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

2. Materials and methods

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

2.1. Animals and housing

A total of 80 healthy Yorkshire castrated male pigs (barrows; 46.5 ± 8.6 kg test day body weight) divergently selected for RFI were used. Half (20 low-RFI and 20 high-RFI) of the pigs were fed a low-fiber, high-energy diet (9.4% neutral detergent fiber, 13.86 MJ of metabolizable energy/kg of feed) and half were fed a high-fiber, low-energy diet (25.9% neutral detergent fiber, 11.97 MJ of metabolizable energy/kg of feed). Both diets met or exceeded NRC (1998) requirements and further information regarding ingredient and nutrient analysis is explained by Colpoys and colleagues (2014). Two genetic line treatments were compared: low-RFI (n=40) and high-RFI (n=40). Divergent line selection criteria were based on estimate breeding values for RFI as explained by Cai and colleagues (2008). The low-RFI genetic line had been selected over eight generations whereas the high-RFI genetic line had been randomly selected over five generations, and then selectively bred for high-RFI over the next three generations.

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

2.2. Test methodology and facility

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

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

The HAT and NOT were conducted in a rectangular arena separate from the home pens. The arena setup was adapted from published work by Hemsworth and colleagues (1989) and Marchant-Forde and others (2003). The arena measured 4.9 m long x 2.4 m wide and had 1.2 m high, black corrugated plastic sides that were attached to gates. In order to hide the human observer visually during NOT, a 1.2 m wide x 2.2 m high black corrugated plastic observation hide was positioned outside the arena. Concentric curves were drawn on the slatted concrete floor using permanent marker one day before the start of testing to divide the arena into four zones in order to measure the location of the pig in proximity to the novel stimulus. Zone 1 was defined as oral, nasal, and/or facial contact with the human or the cone during HAT and NOT, respectively. For consistency with the other zones, pigs that touched the human or the cone will be referred to as entering zone 1. Zone 2 was the area nearest to the novel stimulus and zone 4 was the area where the pig entered the test arena, furthest from the novel stimulus. Zones 2, 3, and 4 consisted of approximately equal area which allowed the entire body of the pig to fit within the zone. The concentric curves allowed each zone to measure a consistent distance from the novel stimulus (Fig. 1). Located in the center of the arena, 2.3 m from the ground, was one electronic recording device (HOBO Pro v2, temp / RH, U23-001, Onset Computer Corporation, Bourne, MA, USA) that recorded ambient temperature (°C) and relative humidity (%) every 5 min for the duration of the testing. Throughout the testing period, the mean (±S.D.) ambient temperature was 15.3 (±2.2)°C, 6.4°C cooler than the home pen, and relative humidity was 73.2 (±10.6)%, 2.7% higher than the home pen.

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

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

2.2.1. Human approach test

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

2.2.2. Novel object test

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

2.3. Measures

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

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

2.4. Data analysis

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

3. Results

3.1. Human approach test

3.1.1. Stimulus attention

Low-RFI pigs took 48 s longer to enter zone 1 compared to the high-RFI pigs ($P = 0.04$). No differences were observed between lines for total number of zone 1 entrances (P = 0.16) or duration of time spent within zone 1 (P = 0.93; Table 2). Duration 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 ($F_{1,63}$ = 0.04, P = 0.85) did not differ between lines. Furthermore, no line differences were observed in duration of time spent with head in front ($F_{1,63}$ = 0.2, P = 0.63), side ($F_{1,63}$ = 2.4, P = 0.13), or back ($F_{1,63}$ = 0.7, P = 0.40) orientation relative to the human.

3.1.2. Arousal and fear behavior

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

3.2. Novel object test

3.2.1. Stimulus attention

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

3.2.2. Arousal and fear behavior

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

4. Discussion

4.1. Stimulus attention

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

One explanation is that the high-RFI pigs were more fearful of social isolation than the human or cone. Social isolation can be distressing for pigs (Gonyou, 2001) and is likely one of the greatest stressors during HAT and NOT (Forkman et al., 2007; Pairis et al., 2009). A second explanation is that approach latency reflects coping style rather than the level of fear. Hayne and Gonyou (2006) proposed that a fast approach is indicative of an active response whereas a slow approach is indicative of a passive response in pigs. This interpretation is further supported by Hessing and colleagues (1994), who reported that pigs quicker to approach a novel object were also more resistant to a back test. These two explanations should be considered in future research.

4.2. Arousal and fear behavior

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

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

4.3. General discussion

Preliminary analysis of the HPA axis in these lines of pigs reported that low-RFI gilts tended to have lower cortisol concentrations both before and after an exogenous adrenocorticotropin hormone challenge compared to high-RFI gilts (Jenkins et al., 2013). Therefore, the reduced behavioral reactivity seen in low-RFI pigs compared to high-RFI pigs may in part be due to a reduced physiological stress response. Furthermore, eighth generation, high-RFI pigs from first parity sows (in contrast to pigs from second parity sows investigated in the current study) had 241 g/d greater RFI and 2.5 mm greater backfat than low-RFI pigs (Young and Dekkers, 2012). Reduced feed efficiency and increased carcass fat has been observed in stressed pigs within the commercial environment (Black et al., 2001); therefore, may suggest greater stress in high-RFI than low-RFI pigs within the commercial home pen environment.

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

5. Conclusions

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

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References

Amdi, C., Williams, A.R., Maloney, S.K., Tauson, A.H., Knott, S.A., Blache, D., 2010. Relationship between behavioural reactivity and feed efficiency in housed sheep. Animal Production Science 50, 683-687.

Barea, R., Dubois, S., Gilbert, H., Sellier, P., van Milgen, J., Noblet, J., 2010. Energy utilization in pigs selected for high and low residual feed intake. Journal of Animal Science 88, 2062-2072.

Black, J.L., Giles, L.R., Wynn, P.C., Knowles, A.G., Kerr, C.A., Jones, M.R., Strom, A.D., Gallagher, N.L., Eamens, G.J., 2001. A review - Factors limiting the performance of growing pigs in commercial environments, 8th Biennial Conference of the Australasian Pig Science Association, Adelaide, Australia, pp. 9-36.

Boddicker, N., Gabler, N.K., Spurlock, M.E., Nettleton, D., Dekkers, J.C.M., 2011a. Effects of ad libitum and restricted feed intake on growth performance and body composition of Yorkshire pigs selected for reduced residual feed intake. Journal of Animal Science 89, 40-51.

Boddicker, N., Gabler, N.K., Spurlock, M.E., Nettleton, D., Dekkers, J.C.M., 2011b. Effects of ad libitum and restricted feeding on early production performance and body composition of Yorkshire pigs selected for reduced residual feed intake. Animal 5, 1344-1353.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Hemsworth, P.H., Barnett, J.L., Coleman, G.J., Hansen, C., 1989. A study of the relationships between the attitudinal and behavioural profiles of stockpersons and the level of fear of humans and reproductive performance of commercial pigs. Applied Animal Behaviour Science 23, 301-314.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Measure	Description
Approach	
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
Head orientation	
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
Elimination	
Urination (n)	Excreting urine
Defecation (n)	Excreting feces
Escape attempt (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 ≥3 s. Duration was measured from the start of the freeze to any movement of the body

Table 2. Latency (s), frequency (n), and duration (%; least square means ± SE) of behaviors during the human approach test in castrated male pigs selected for low-RFI (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 ± 19.67	84.5 ± 12.47	4.5	0.04
Zone 1, n	6.5 ± 0.55	7.4 ± 0.60	2.1	0.16
Zone 1, %	10.6 ± 1.49	10.4 ± 1.47	0.01	0.93
Zone crossings, n	40.6 ± 1.71	48.9 ± 1.99	26.6	<0.0001
Head movements, n	86.7 ± 1.80	92.2 ± 1.87	6.0	0.02
Urination, n	0.5 ± 0.11	0.5 ± 0.12	0.1	0.72
Defecation, n	3.4 ± 0.30	4.9 ± 0.36	10.5	0.002
Escape attempt, n	0.4 ± 0.14	1.1 ± 0.30	11.3	0.001
Escape attempt, %	0.1 ± 0.04	0.2 ± 0.07	3.3	0.08
Freeze, n	4.9 ± 0.65	7.5 ± 0.96	22.8	<0.0001
Freeze, %	4.1 ± 0.71	6.2 ± 0.93	4.1	0.05

Table 3. Latency (s), frequency (n), and duration (%; least square means ± SE) of behaviors during the novel object test in castrated male pigs selected for low-RFI (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	128.1 ± 20.84	76.3 ± 12.31	5.3	0.02
Zone 1, n	7.3 ± 0.63	8.3 ± 0.70	2.4	0.13
Zone 1, %	9.2 ± 1.46	9.1 ± 1.42	0.01	0.93
Zone crossings, n	40.1 ± 1.74	48.4 ± 2.03	28.5	<0.0001
Head movements, n	80.5 ± 2.28	86.2 ± 2.39	7.2	0.01
Urination, n	0.6 ± 0.12	0.5 ± 0.11	0.3	0.60
Defecation, n	3.4 ± 0.30	4.4 ± 0.35	4.7	0.03
Escape attempt, n	0.6 ± 0.18	0.9 ± 0.23	1.6	0.21
Escape attempt, %	0.1 ± 0.05	0.2 ± 0.07	0.9	0.35
Freeze, n	4.7 ± 0.66	7.2 ± 0.96	20.5	<0.0001
Freeze, %	3.9 ± 0.72	6.0 ± 0.92	3.9	0.05

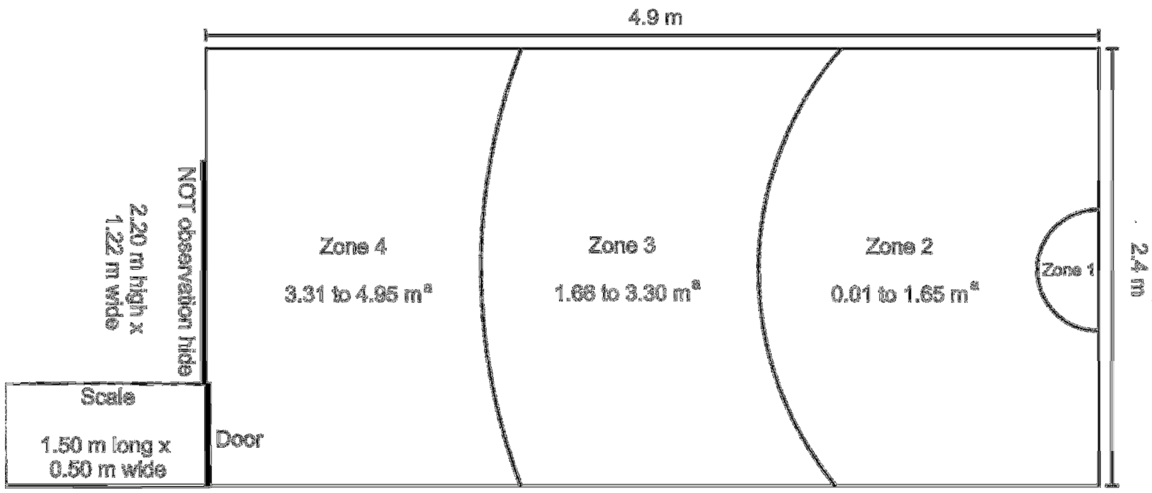

Figure 1. Arena where pigs were tested using human approach- (HAT) and novel object tests (NOT).

[a]Indicates the distance of each zone from the human or cone, located in zone 1. Zones 2, 3, and 4 consisted of approximately equal area.