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Differences in Open Field Behavior Between Heterozygous and Homozygous Negative Gilts for the RYR(1) Gene

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A test widely used to assess fear and novelty responses in domestic species is the open field. The aim of this study was to investigate the effect of RYR(1) genotype on open field behavior in growing pigs. The study subjected 15 heterozygous (Nn) and 15 RYR(1)-free (NN) gilts of 19 weeks of age to 3 replicates of an open field test 2 days apart from each other. The study measured the number of grid lines crossed and defecation score in the test arena. There was a significant individual correlation among the 3 replicates of the test, both for number of grid lines crossed and defecation score ($p <$

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.05). RYR(1) genotype had a significant effect on number of grid lines crossed, with NN gilts showing more overall activity than Nn gilts ($p < .05$). The study observed no significant differences in defecation score between genotypes. This result suggests that the RYR(1) genotype may have an effect on the appraisal of novelty. Thus, it would be interesting to take this factor into account when using this methodology to assess fear responses in pigs and in interpreting the results with respect to welfare.

The RYR(1) gene (n), also known as the Halothane Gene, has been widely associated with a genetic susceptibility to suffer stress because stressful stimuli are likely to trigger a potentially lethal condition known as malignant hyperthermia (MH), especially in homozygous positive (nn) pigs (Cassens, Marple, & Eikelenboom, 1975; Webb, Cardin, Smith, & Imlah, 1982).

Both heterozygous (Nn) and homozygous positive pigs present a mutation in the RYR(1) gene, which encodes the skeletal muscle ryanodine receptor (Fujii et al., 1991). The mutation is linked to an abnormal regulation of the Ca^{2+}-release channel, thereby inducing muscle contraction, hypermetabolism, and hyperthermia (O'Brien, Klip, Britt, & Kalow, 1990). Important research efforts have been directed toward understanding the physiology of these pigs (Marple & Cassens, 1973; Schaefer, Doornenbal, Tong, Murray, & Sather, 1987) and the consequences of the gene on production and economics (Fisher, Mellett, & Hoffman, 2000; Gispert et al., 2000; Larzul et al., 1997) as well as toward defining predictive tests to identify the three RYR(1) genotypes (Fujii et al., 1991).

However, few studies have attempted to evaluate the welfare of these pigs using indicators such as behavior in a social or nonsocial context. Some investigations have reported differences in some behavioral patterns (Robert & Dallaire, 1986; Schaefer, Sather, Tong, & Lepage, 1989), cortisol or creatin-phospho kinase increases after stressful situations (Fàbrega et al., 2002; Geers et al., 1994; Gispert et al., 2000), or sympatho-adrenomedullary functioning (Gregory & Wotton, 1981) between genotypes. According to other studies (Schaefer et al., 1989), heterozygous and homozygous negative pigs show few differences in behavior if observed in a normal social context. Nevertheless, no data are available to determine whether the behavior of these two types of pigs differs when subjected to a presumably more challenging situation like the open field (OF) test.

The OF test originally was developed for laboratory animals (Gray, 1987; Hall, 1936; Whimbey & Denenberg, 1967) but has been used or modified to assess fear-related responses or reactions to novelty in livestock species such as pigs (Andersen, Bøe, Foerevik, Janczak, & Bakken, 2000; von Borrell & Ladewig, 1992), cattle (Boissy & Bouissou, 1995), and sheep (Romeyer & Bouissou, 1992). Behavior in an OF is determined mainly by the conflict between exploration of and aversion to a new environment (Asano, 1986). The traditional interpretation of the OF data in rodents has been that a high defecation rate and low activity indicate a high level of fear or emotionality (Broadhurst, 1957; Denenberg, 1964).

However, several authors have pointed out the difficulty in interpreting the results of an OF when adapted for domestic, nonhuman animals. In this sense, it has been argued that OF behavior may be affected by factors apart from the animal's appraisal of novelty, such as rearing experience, genetic differences, or social motivation (Boissy & Bouissou, 1995; von Borrell & Ladewig, 1992). In pigs, an effect on OF behavior of factors such as sex and breed (Mormède, Dantzer, & Bluthe, 1984), age at weaning (Dantzer & Mormède, 1981), environmental enrichment and rearing conditions (Beattie, Walker, & Sneddon, 1995), and neonatal handling (Weaver, Aherne, Meaney, Schaefer, & Dixon, 2000) has been described. However, as mentioned previously, the RYR(1) genotype of pigs has not been taken into account as a factor that could influence the results of the test.

Because the RYR(1) genotype has been associated with a proneness to suffer stress, this experiment attempted to determine whether RYR(1) Nn and NN pigs behave differently when subjected to an OF test. In addition, the experiment aimed at determining whether the RYR(1) genotype could be of importance when using OF tests to assess appraisal of novelty. Thus, the hypothesis underlying the experiment was that Nn pigs would be more reactive when confronted with a novel environment and that this would be reflected in their behavior in the OF test.

There were three replicates of the test to analyze whether there was an effect of habituation and whether it differed between Nn and NN pigs. To simplify the factors that could influence the interpretation of the results, only females were tested. Because homozygous positive pigs represent a lower percentage of Spanish slaughter pigs than homozygous negative or heterozygous pigs, the genotypes compared—RYR(1) heterozygous and homozygous negative pigs—reflected commercial conditions in Spain. In some European Union countries such as Denmark, RYR(1) gene frequency has been decreased to promote better welfare and meat quality. In some other countries, however, there is a high demand for well-conformed carcasses. This is linked closely to a major incidence of the RYR(1) gene (in Spain a recent survey of five different slaughterhouses found that the frequency of the n allele varied from 8% to 54% depending on the abattoir; Gispert et al., 2000).

MATERIAL AND METHOD

Experimental Housing and Animals

The experiments were conducted on a commercial farm in early June 2001. The gilts observed were crosses of Large White × Landrace RYR(1) free (NN) sows with a Piétrain heterozygous (Nn) terminal sire line. Eight boars of the paternal line were used for the inseminations, which were carried out within a week. The RYR(1) genotype of the sows and sires used was determined from a hair sample using PCR (polymerase chain reaction) amplification and digestion with restriction enzymes as described by Fujii et al. (1991). Each gilt observed was chosen randomly from 30 different litters (the experiment was part of a larger investigation in which 80 litters were controlled and 1 male and 1 female piglet from each litter were selected).

The animals were born in conventional farrowing crates, and eye teeth and tails were clipped between 2 and 4 days of age. Tail samples were used to determine the RYR(1) genotype of the gilts using the same technique described previously. Piglets were weaned at 3 weeks and moved to a transition farm. At 9 weeks, pigs were moved to the fattening pens with partly slatted concrete floors, where they were kept in groups of 10 until they were slaughtered at 25 weeks of age. Five females of each genotype were assigned to the three OF experimental groups (three groups of 10 individuals each). Space allowance was 1.1 m^2 per pig. Two of the home pens of these three groups were located opposite one another, and the third one was contiguous to one of these two. The animals were fed *ad libitum* a standard pelleted growers' food (contents per kg fresh: 177 g crude protein, 14 MJ digestible energy) by hand twice a day, at 0800 and 1600 hr. They had free access to water from a nipple drinker. Room temperature ranged from 20° to 27°C.

OF Test

The 15 NN and 15 Nn gilts were tested at 19 weeks of age. Each gilt was subjected to three replicates of the test 2 days apart from each other. All tests were performed between 0800 and 1000 hr on 9 consecutive days. Thus, the 10 gilts (5 Nn and 5 NN) in Group 1 were tested on Days 1, 4, and 7; gilts in Group 2 on Days 2, 5, and 8; and gilts in Group 3 on Days 3, 6, and 9. The subjects were tested individually for 5 min in a test room located in the same building but in auditory, visual, and olfactory isolation from the home pen. The farm building was partitioned midway by a solid wall to create two rooms, one of which contained the OF pen and the other of which contained the home pens.

The distance from the home pens to the OF pen was approximately 30 m for two of the groups and 32 m for another group. The test room was surrounded by concrete walls on three sides, a concrete wall with a door at the other end, and measured 4.5 × 3.5 m^2 with 25 equal-sized squares (0.8 m × 0.8 m) painted on the floor. To make the grids clearly visible, the number of squares was chosen due to the type of slatted floor. A video camera was suspended above the center of the arena, and recording commenced before each pig was introduced to the pen.

After the gilt was released in spot number 1, an observer in a neighboring room—out of sight of the gilt—also recorded the following behavioral patterns: number of lines crossed (i.e., both forelimbs crossed a line), occurrence of defecation (separated by > 10 sec, criterion previously used by Jones & Nicol, 1998), and latency to eat. Latency to eat was included in the OF to investigate whether feeding behavior was inhibited as a consequence of stress and, in this case, to see whether there was a difference between NN and Nn pigs in this response. The food provided was the same that the gilts ate at their home pen. It was placed in a food trough located in the same corner as the one the gilts had in their home pen. Water was available throughout the test from a nipple drinker.

After the test the gilts were led back to their home pens. Two people always handled and gently pushed the animals to the test pen if they were not willing to move voluntarily from or to the home pen. The arena was cleaned between each test. Video recordings were used to assess the reliability of direct observations. Both recording systems were found to correlate ($p < .05$).

Statistical Analysis

Prior to analysis, data were subjected to a square root transformation (Martin & Bateson, 1993). Individual consistency across the three replicates of the OF tests was assessed by calculating Pearson correlations of the number of lines crossed and defecations (interreplicate correlation). In addition, the Pearson correlation between number of defecations and lines crossed within each replicate was determined (intrareplicate correlation). Latency to eat was not considered in the analysis because most of the animals did not perform this behavior.

The effect of RYR(1) genotype on OF behavior was assessed by means of a repeated measures model using PROC MIXED with the SAS computer software (SAS for Windows, Version 8.1, 1999–2000). The fixed factors initially included in the model were RYR(1) genotype, group, replicate, and their interactions. The covariance structure chosen was the Autoregressive (AR) (1) because it was the one that best fit the model according to Schwarz's Bayesian Criterion. Group and interactions did not affect significantly the two variables studied and were removed from the final statistical model.

RESULTS

Pearson interreplicate correlations were found to be significant ($p < .05$) for all paired comparisons for both number of lines crossed and defecations (Table 1). Conversely, intrareplicate correlations (number of grid lines crossed vs. defecations) were not significant: $r = .26$, $r = .12$, and $r = –.35$ for Replicates 1, 2, and 3, respectively.

Means and standard errors of the parameters recorded during the three replicates of the OF test are shown in Table 2. According to the repeated measures model applied, RYR(1) genotype had a significant effect on number of grid lines

TABLE 1
Interreplicate Pearson Correlations for Number of Grid Lines Crossed and Defecations

		Grid Lines Crossed		*Defecations*	
		Test 2	*Test 3*	*Test 2*	*Test 3*
Grid lines crossed	Test 1	.63**	.47**		
	Test 2		.48**		
Defecations	Test 1			.83***	.41*
	Test 2				.46*

*p < .05. **p < .01. ***p < .001.

TABLE 2
Mean Values (With *SE*) of the Parameters Recorded During the Open Field Tests for the Two RYR(1) Genotypes

Data Category	*Test 1*		*Test 2*		*Test 3*		*Overall Significance*
	NN	*Nn*	*NN*	*Nn*	*Nn*	*Nn*	
No. of grid lines crossed[a]							
M	69.8_a	60.9_a	79.7_a	62.4_b	68.1_a	57.4_a	.035
SE	7.09	4.78	7.82	3.73	5.15	6.76	
No. of defecations[a]							
M	1.5_a	1.4_a	1.5_a	1.5_a	2.0_a	1.5_a	*ns*
SE	0.45	0.24	0.36	0.27	0.40	0.25	

Note. Values with different subscripts within the same replication differ at $p < .05$.
[a]Mean values of the number of lines or defecations/pig/5-min test.

crossed ($p < .05$), with NN gilts crossing the grid lines more times than Nn gilts in all tests and significantly more times in Test 2. On the contrary, frequency of defecations did not differ significantly between genotypes. Replicate did not significantly affect either number of grid lines crossed or defecation. This is in agreement with the correlations found among the three replicates mentioned previously.

DISCUSSION

The OF tests of this study were carried out to assess whether there were differences in responses to novelty of pigs differing in their RYR(1) status. The results obtained for the numbers of grid lines crossed suggest that there are individual differences between Nn and NN pigs when challenged by a presumably frightening or stressful situation. Behavior of an individual in an OF test results from the conflicting motivations between exploration and fear aversion of a new environment (Asano, 1986).

According to the traditional interpretation of this test with laboratory animals (Broadhurst, 1957), a higher level of fear or emotionality would be associated with less overall activity ("freezing") and a greater defecation score. Such interpretations have been questioned, however, because both freezing and "active escape" are expressions of emotional behavior (Archer, 1973); thus, understanding the motivation underlying the different behavioral patterns recorded during the test plays is important to draw conclusions.

In pigs, the interpretation of the OF also has been contradictory. Some investigations (Spoolder, Burbidge, Lawrence, Simmins, & Edwards, 1996) have found that the largest proportion of the variance was explained by behavior related to fear, whereas other studies have described exploration activity as the major component of the variance (Andersen et al., 2000; Jensen, Rushen, & Forkman, 1995). These differences have been attributed to factors such as sex or breed (Mormède et al., 1984), rearing conditions (Beattie et al., 1995), or a certain age dependency in exploratory behavior (Wood-Gush, Vestergaard, & Volker-Petersen, 1990). These results suggest that to some extent the RYR(1) genotype also could explain the contradictory results found in the studies mentioned previously, as this factor has not been controlled in most of the OF experiments with pigs. This study tried, as explained earlier, to control factors that could influence the results—sex, test order, distance to test pen or maternal litter—so that the RYR(1) genotype can be assumed to explain most of the differences observed between NN and Nn gilts. In this respect, pigs selected for high levels of lean gain (a carcass trait usually higher among RYR(1) carriers compared to RYR(1)-free pigs) showed lower levels of activity in an OF test than pigs selected for low levels of lean gain (Shea-Moore, 1998), even though straightforward comparisons with this study are not possible because the pigs' RYR(1) genotype was not determined in Shea-Moore's (1998) experiment.

A potential explanation for the differences between Nn and NN pigs could be attributed to the physiological response to stress. Disturbed cortisol levels (very low or very high) have been found to influence the behavioral reactivity to novelty in rats (Oitzl, Fluttert, & Kloet, 1994), and this relation also may exist in pigs (de Jong et al., 2000; von Borrell & Ladewig, 1992).

Other investigations (Fàbrega et al., 2002; Marple & Cassens, 1973) have found differences in cortisol rises in pigs of different RYR(1) status after stressful situations. Further research would be required to test the hypothesis that the differences in OF behavior between different RYR(1) genotype pigs are associated with adrenocortical functioning.

The values found for number of grid lines crossed and defecation score fall into the intervals reported by previous workers. Grid lines crossed varied from 38 to 135.4 and defecation scores ranged from 1.6 to 3.5 (Jones & Nicol, 1998; von

Borrell & Ladewig, 1992). These differences between studies may be associated with methodological aspects like length of the test or design of the test pen. Findings in other studies support the lack of correlation reported here between defecation and locomotor activity (Andersen et al., 2000; Hutson, Amborse, Barnett, & Tilbrook, 2000; von Borrell & Ladewig, 1992). Hutson et al. (2000) found that elimination in OF tests failed to discriminate between different aversive stimuli, and Andersen et al. (2000) also reported no correlation between the defecation score and locomotion or the rotated factors in their principal component analysis (fear of novelty or activity).

For this reason, Anderson et al. (2000) suggested that defecation in pigs might not represent a general response to frightening situations in the same manner as for rodents. The smaller defecation frequency in the OF compared to rodents, the loss of feces on the way to the test arena, or nutritional factors might limit the validity of this measurement for pigs. In our study, the latency to eat variable was not useful because the gilts rarely performed that behavior, and often it was difficult to determine if animals actually were eating when their heads were in the food boxes. This agrees with the findings of Hutson et al. (2000), who reported similar methodological constraints and considered this variable a poor measure for evaluating responsiveness to novel stimuli compared to approach or avoidance distance.

Literature on consistency of replicates of tests and on intertest correlations also shows conflicting results, which have been attributed to differences in the stability of the underlying motivations of the behavioral and physiological reactions to a certain test (Erhard, Mendl, & Christiansen, 1999; Ruis et al., 2000). Because perception of novelty might change—due not only to habituation but also to aging—the time span between subsequent tests may be, to some extent, one of the explanations for these conflicting results.

The three replicates in this study were 2 days apart. In agreement with studies with a similar time span (Hessing, Hagelsø, Schouten, Wiepkema, & van Beek, 1994; Jensen et al., 1995), a significant correlation was found between them for both genotypes and both parameters recorded.

WELFARE IMPLICATIONS

That the differences in locomotion observed between the two RYR(1) genotypes in the OF test were significant and consistent across the three replicates has methodological and on-farm implications. On methodology grounds, it would be interesting to consider this factor when using this test to assess fear in pigs because it might interact with other factors and produce confounding results.

During the last decade, the study of individual behavioral differences in coping styles in pigs has received much attention, in an attempt to provide information on the selection of animals that are better adapted to their environment and, thereby, enhance their welfare (Erhard et al., 1999; Jensen et al., 1995; Ruis et al., 2000; Thodberg, Jensen, & Herskin, 1999). Taking into consideration the RYR(1) genotype of the experimental pigs could help to interpret those results. In relation to on-farm implications, these differences in OF behavior might indicate that Nn pigs are more susceptible than are NN pigs to stressful stimuli and might respond differently to novel situations than do NN pigs. Thus, in contexts such as transportation or introduction to a new environment, a different welfare status would be expected for NN and Nn pigs.

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