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Original article

Behavioural patterns and performance in heterozygous or halothane free suckling piglets and growing gilts

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Abstract – The aim of this study was to compare the behaviour of halothane free pigs (NN) and heterozygous pigs (Nn) when they are raised under standard commercial conditions. A total of 110 suckling piglets (50 Nn and 60 NN) and 80 growing gilts (40 Nn and 40 NN) were observed in their farrowing or fattening pens. The observations were carried out in two trials (51 piglets and 40 gilts in trial 1 and 59 piglets and 40 gilts in trial 2). Instantaneous scan sampling was used to record the frequencies of resting, non-sucking activity, sucking/eating or interactions between individuals. The piglets and gilts were weighed one day after birth, at 20 and 180 days of age and average daily gain was estimated. The halothane genotype had, in general terms, a small and inconsistent effect on the behavioural patterns recorded for the observations of the piglets and gilts observations (i.e. both genotypes were seen to have similar frequencies for the behavioural patterns considered). No differences in body weight or average daily gain were found either between NN and Nn piglets or gilts. These results suggest that when pigs are raised under conventional commercial groups, the behaviour of NN and Nn pigs would be more affected by environmental factors such as the time of day or age than the halothane genotype.

RYR(1) genotype / pig – social behaviour / performance / stress

Résumé – Comportement social et performances de croissance des porcelets allaités et des porcs en croissance en fonction de la présence du gène de sensibilité à l'halotane. L'objectif de cette étude était de comparer le comportement de porcelets allaités et de truies en croissance, hétérozygotes (Nn) ou homozygotes (NN, sans le gène RYR(1)), dans des conditions d'élevage standard. Un total

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de 110 porcelets allaités (50 Nn et 60 NN) et 80 femelles en croissance (40 Nn et 40 NN) ont été observés dans leurs conditions normales d'élevage. Les observations ont été réalisées en deux essais (51 porcelets et 40 femelles en croissance dans l'essai 1 et 59 porcelets et 40 femelles en croissance dans l'essai 2) et dans quatre unités de production. La méthode de Scan-sampling a été utilisée pour enregistrer les fréquences de repos, l'activité de non tétée, la tétée/alimentation ou les interactions entre les individus. Le poids vif des porcelets et des femelles en croissance a été mesuré à 1, 20 et 180 jours d'âge et le gain de poids vif journalier a été calculé. De façon générale, le génotype RYR(1) a un effet faible et variable sur les comportements enregistrés tant chez les porcelets que chez les femelles en croissance. Le génotype RYR(1) n'a pas une incidence significative sur le poids corporel des porcelets ou des femelles en croissance dans les deux essais. Ces résultats suggèrent qu'avec des méthodes d'élevage communes, le comportement social des porcs charcutiers NN et Nn présente des différences minimes, et d'autres facteurs environnementaux tels que l'âge ou l'heure de la journée influencent de façon plus marquée le comportement social.

génotype RYR(1) / porc charcutier – comportement social / performance de croissance / stress

1. INTRODUCTION

Stress is a common phenomenon in social animals, especially if resources are limited. Changes in behaviour have been said to be one of the most obvious indicators that an animal is having difficulty coping with environmental and social challenges [2]. In pigs, the halothane gene (n) has been widely associated with a high susceptibility to develop stress, since stressful stimuli are likely to trigger a potentially lethal condition known as malignant hyperthermia (MH) in homozygous positive pigs (nn). When the DNA genetic probe for the halothane gene became commercially available after the findings of Fujii et al. [7], the debate for commercial producers was focused on whether to use halothane carrier boars (Nn) or to select halothane free lines. New lines of investigation have emerged to determine the characteristics of Nn pigs obtained from such crosses. This research has concluded that nn and Nn individuals present significantly higher mortality rates during transport and lairage [11] and poorer meat quality associated with a higher incidence of Pale, Soft and Exudative meat (PSE) than NN pigs [8], as well as some differences in productivity [3, 10]. In addition, some authors have reported differences in stress physiology [8] and a potential effect of the gene on behaviour has also been suggested [13]. However, few studies have attempted to evaluate whether the three halothane genotypes (i.e. distinguishing Nn and NN pigs) clearly differ in their behaviour under the conventional groups in commercial farms.

Although originally the term "stress" was used to refer to the physiological responses involved in adaptation to the environment, nowadays the term usually refers to the animal's state when it is challenged beyond its behavioural and physiological capacity to adapt to its environment [6]. Thus, stress is generally associated with a compromise in animal welfare. Therefore, if behaviour of NN and Nn pigs was found to differ consistently, this could be an indicator of a welfare compromise.

The present study is part of a bigger investigation which is aimed at elucidating the benefits or disadvantages of eliminating the halothane gene from Spanish breeding schemes, taking into account welfare, productivity and meat quality aspects. The present paper summarises the results comparing the behaviour of Nn and NN pigs. The experiments were designed to resemble commercial conditions, thus, two trials were carried out to compare heterozygous and halothane free pigs obtained from two of the commercial alternatives of major interest at present for pig breeders (i.e. heterozygous or halothane free boars crossed with halothane free sows).

2. MATERIALS AND METHODS

2.1. Experimental housing and animals

The experiments were conducted at four commercial farms belonging to the same pig company and consisted of two trials of observations in farrowing and fattening pens (first trial from August 2000 to January 2001 and second trial from January 2001 to July 2001). In the first trial, the pigs observed were crosses of Large White × Landrace halothane free (NN) sows with two heterozygous (Nn) terminal sire lines: a Large White × Pietrain and a Pietrain. In the second trial, the same sows (when possible) were selected but crossed with two different terminal sire lines: a Pietrain homozygous positive (Pi nn) and a Pietrain homozygous negative (Pi NN). Eight boars of each line were used for the inseminations that were carried out within a week in both batches. The rest of the experimental set up was identical for both trials.

The piglets were born in conventional farrowing crates with slatted floors, a heat source and a nipple drinker. Between 2 and 4 days of age, eye-teeth and tails were clipped. Tail samples were used to determine the halothane genotype of the offspring, using PCR amplification and digestion with restriction enzymes as described by Fujii et al. [7]. Since sows and boars were all homozygous in the second trial, the halothane genotype of the offspring only needed to be confirmed with a random sample from 30 piglets. On the first day of age, 8 litters (out of 25 litters per farm which were used for the broader investigation) were selected and, from those, 8 piglets (4 males and 4 females) per litter were individually identified to be observed at their farrowing pens according to the protocol described in Section 2.2. A total of 51 individuals (21 Nn and 30 NN) were observed in the first trial and 59 (29 Nn and 30 NN) in the second trial in the farrowing pens. Data was removed for individuals not identified genetically and for piglets that died during the experiments.

Piglets were weaned at 3 weeks of age and moved to a transition farm. At 9 weeks of age, the pigs were moved to the fattening pens with partly slatted concrete floors, where they were kept in groups of 10 animals until they were slaughtered at 25 weeks of age. There, 20 Nn and 20 NN gilts were selected and randomly assigned to four experimental groups (i.e. 2 groups of Nn and 2 groups of NN gilts). One of those groups of gilts had already been observed at the farrowing pens and the other group was made up of new animals selected from the pigs controlled as part of the bigger investigation. Only gilts were observed to eliminate one factor of variation. Space allowance was 1.1 m^2 per pig. The animals were fed ad libitum a standard pelleted growers feed (containing per kg fresh: 177 g crude protein, 14 MJ digestible energy) by hand twice a day, at 08.00 h and 16.00 h. They had free access to water from a nipple drinker. Room temperature ranged from 20–27 ºC. Natural light at a minimum of 40 lux was available for observations carried out during the winter from 08.30 h to 16.30 h and from 07.30 h to 18.30 h for the observations carried out during the summer. Lights were on from 16.30 h to 17.30 h for the winter observations, which lasted up to 17.00 h.

The observed piglets were weighed one day after birth and at 20 days of age (on the day before weaning) and the gilts at 180 days of age (two days before slaughter).

2.2. Behavioural measurements

The behaviour of the animals in the farrowing and fattening pens was observed directly using instantaneous scan sampling. The sample intervals were of 1 min for the observation of the piglets and 3 min for the gilts.

For the piglet observations, each litter was observed during a total of 12 hours in both trials, distributed in two morning (09.00 h to 12.00 h) and two afternoon (14.00 h to 17.00 h) observations of 3 hours

Table I. Ethogram showing the behavioural categories of the observations of the piglets and gilts.

Behaviour	Definition
Common behaviours	
Lying	Lying on side or sternum
Standing	Standing on four legs without movement
Walking	Lifting at least three extremities
Running	Trotting, galloping through the pen
Sitting	Standing on fore-legs, hind quarter on the floor
Exploring	Sniffing, rubbing, touching the walls or ground of the pen
Other	Piglet/gilt unsighted or behaving differently from above
Specific piglets' behaviours	
Interacting with piglet	Contact with another piglet (nudging, pushing, biting, playing)
Sucking	From the quiet phase after the piglets stopped massaging the udder and began sucking with slow movements and the rapid phase which corresponds to milk flow
Active at udder	Nudging udder, scrambling from teat to teat or moving around 25 cm of udder
Specific gilts' behaviours	
Agonistic interactions	Performing an aggressive (i.e. biting or replacing another gilt) or submissive (i.e. being replaced or bitten) act towards another gilt
Non-agonistic interactions	Performing a neutral act towards another gilt (i.e. nudging, nose-nose contact, nose-body contact)
Eating	Head in the feeder

each. The observations were carried out during 16 consecutive days, rotating the 8 litters so that all of them had been observed 2 mornings and 2 afternoons (i.e. there were 4 days between the observations of one litter. For example, litter L was observed on days 1, 5, 9, 13 and the same patron for the other litters). The age of the piglets during the observations ranged from 3 days to 19 days.

Concerning the gilt observations, in the first trial, each group of gilts was observed for 32 hours, distributed in 16 hours of morning observations (08.00 h to 12.00 h) and 16 hours of afternoon observations (13.00 h to 17.00 h). Therefore, each group had 4 morning and 4 afternoon observations of 4 hours each. However, the gilts had to be treated because of a respiratory problem just before the last observation, thus, these data were discarded. These observations were carried out during two consecutive days per week over 8 consecutive weeks. In the second trial, the observations were carried out during 4 consecutive days per week over 4 weeks, one week apart from each other. As for the piglet observations, the observations of the four groups were rotated so that all groups had the same number of observations in each period. The age of the growing gilts during the observations was from 75 to 135 days old.

The ethogram defined to record the behaviour is summarised in Table I, and was

based on those used by Blackshaw et al. [1] concerning the behaviour of the piglets and on Jensen [9] and Schaefer et al. [13] in relation to the behaviour of the gilts. Mean frequencies of occurrence of the behavioural categories defined were calculated for each individual and genotype. Frequencies were chosen to monitor behaviour since they have been traditionally used to determine and compare time budgets in behaviour studies. The behavioural categories were reduced to main categories for the analysis. Concerning the ethograms of the piglets, three main categories were defined: non-sucking activity (including walking, running, interacting with another piglet or the pen or active at udder), resting (standing, lying or sitting) and sucking. The behaviour of the gilts was reduced to four general categories: activity (walking, running or exploring/interacting with the pen), resting (standing, lying or sitting), eating and interactions (including agonistic and non-agonistic interactions between animals).

2.3. Statistical analysis

No attempt was made to compare both trials because of the potential effect of environmental conditions (i.e. season of the year and farm conditions). No differences in behaviour between sexes were found for the piglet observations, thus, both sexes were pooled together in both trials. In trial 1, the terminal sire line did not have an effect on behaviour, thus, the comparisons were made between NN and Nn piglets or gilts. In trial 2, since all NN and all Nn piglets or gilts came from a different terminal sire line, comparisons were made between Pietrain NN (Pi-NN) or Pietrain nn (Pi-nn) sired pigs, taking into account that the effect of the halothane genotype would be nested in that of the terminal sire line. Statistical analysis was performed using the computer software Statistical Analysis System (SAS system for windows, version 8.1, 1999–2000). Level of significance was set at $P < 0.05$.

Live weights of both genotypes at 2, 20 and 180 days of age and average daily gains were compared by means of PROC GLM (General Lineal Model). Mean frequencies of occurrence of the behavioural categories defined were calculated for each individual and genotype. In a preliminary analysis, these frequencies were considered to be repeated measurements through time. A PROC MIXED model for repeated measurements, with time, genotype and its interaction as fixed effects was carried out. However, since no consistent effect of genotype through time was observed using this model, an overall mean of the frequencies of each behavioural category for the morning or the afternoon observations was calculated (for example, this overall mean was the mean of the three morning or the three afternoon observations for the growing gilts in trial 1 and that of the 4 morning or 4 afternoon observations in trial 2). Morning and afternoon observations were analysed separately since the time of day of observation proved to have an effect on behaviour. This overall mean was analysed using a PROC GLM (one for morning observations and one for afternoon observations) to evaluate the effect of behaviour on each behavioural category.

3. RESULTS AND DISCUSSION

The two trials of the present experiment were aimed at comparing heterozygous and halothane free pigs obtained from two terminal sire line strategies: heterozygous boars (trial 1) or halothane free and halothane positive boars (trial 2). Due to differences in environmental factors, such as farm or season of year, a direct comparison between both trials was not possible, and, thus, the results were analysed and are presented separately.

3.1. Behaviour

In both trials, either halothane genotype or terminal sire line had a small effect on the

Table II. Mean frequencies (%) of the behavioural patterns of the two halothane genotype (NN or Nn) or terminal sired (Pi NN or Pi nn) suckling piglets observed in the farrowing pens in trials 1 and 2.

Trial 1	Morning observations			Afternoon observations		
	Halothane genotype			Halothane genotype		
Behaviour	NN (n = 30)	Nn (n = 21)	RSD	NN (n = 30)	Nn (n = 21)	RSD
Inactivity	75.4	74.2	6.3	71.3	73.1	5.7
Non-sucking activity	12.4	13.6	5.2	14.6	13.0	5.7
Sucking	9.4	9.2	1.7	10.6	10.3	3.0
Trial 2	Morning observations			Afternoon observations		
	Terminal sire line			Terminal sire line		
Behaviour	Pi NN (n = 30)	Pi nn (n = 29)	RSD	Pi NN (n = 30)	Pi nn (n = 29)	RSD
Inactivity	70.8[a]	74.5[b]	4.3	67.3	71.1	7.2
Non-sucking activity	10.2	11.3	4.1	12.8	12.9	5.01
Sucking	12.9[a]	8.9[b]	3.1	10.3	9.8	2.3

Mean values with different superscripts differ at $P < 0.01$ (comparisons are made between genotypes (trial 1) or terminal sire lines (trial 2) within morning or afternoon observations. Afternoon and morning observations were analysed separately).

behaviour or performance (live weight and average daily gain) of the piglets and gilts. Mean frequencies and standard errors of the behavioural patterns performed by suckling piglets and growing gilts in both trials are summarised in Tables II and III, respectively. The two halothane genotype piglets and gilts differed significantly in several behavioural patterns, but the differences were not consistent along time. For example, the Nn piglets in trial 2 were seen to be more active than the NN piglets ($P < 0.01$), but during the fattening period (i.e. growing gilts), the NN gilts were more active during the morning observations ($P < 0.01$). Moreover, in the first trial, the genotype of the gilts had an effect on agonistic behaviour during the morning observations, with halothane carriers performing a higher frequency of interactions compared to halothane free gilts ($P < 0.01$).

Although the results of Schaefer et al. [13] suggested that there could be some specific differences in behaviour among halothane negative, halothane carriers and halothane positive individuals, the present study does not provide enough evidence to support clear-cut different social behavioural patterns between Nn and NN pigs in conventional farm groups. It is interesting to point out that Schaefer et al. [13] found that the major differences lie between nn and NN pigs, whereas in the present study nn pigs were not included because they represent a low percentage of the Spanish fattening pig population. One possible explanation for that could be related to the genetic role of the n allele in heterozygous pigs, i.e. whether it has a recessive or additive effect on behavioural traits, since the same controversy has been raised for carcass quality traits [14]. However, other experiments carried out with the same pigs [4] showed that differences in responses to novelty or stressful situations like transportation may exist between NN and Nn pigs. Therefore,

Table III. Mean frequencies (s.e.) of the behavioural patterns of the two halothane genotype gilts recorded in trial 1 and the two terminal sired gilts in trial 2.

Trial 1	Morning observations			Afternoon observations		
	Halothane genotype			Halothane genotype		
Behaviour	NN (n = 40)	Nn (n = 40)	RSD	NN (n = 40)	Nn (n = 40)	RSD
Inactivity	73.7	74.4	7.2	59.6	58.3	9.0
Activity	9.8	10.6	4.3	15.9	18.4	5.6
Agonistic interaction	4.7[a]	6.1[b]	1.9	8.8	9.1	2.6
Eating	10.1[a]	7.2[b]	3.1	12.3	10.7	3.4
Trial 2	Morning observations			Afternoon observations		
	Terminal sire line			Terminal sire line		
Behaviour	Pi NN (n = 40)	Pi nn (n = 40)	RSD	Pi NN (n = 40)	Pi nn (n = 40)	RSD
Inactivity	78.6[a]	83.9[b]	4.5	75.6[a]	81.6[b]	6.8
Activity	13.5[a]	7.6[b]	4.0	13.4[a]	8.3[b]	4.6
Agonistic interaction	3.1	2.7	1.2	4.2[a]	2.6[b]	2.2
Eating	3.6	4.3	1.6	4.7	5.6	1.9

Mean values with different superscripts differ at $P < 0.05$ (comparisons are made between genotypes (trial 1) or terminal sire lines (trial 2) within morning or afternoon observations. Afternoon and morning observations were analysed separately).

the lack of effect of genotype on social behaviour in the present study would be more attributable to the fact that behavioural traits result from the additional effects of the environment and genetics. Thus, under the present conditions, environmental factors such as time of day proved to have a more important impact on group behaviour than the halothane background of the individuals. As it has been pointed out in the Materials and Methods section, there were significant differences in the behaviour between the morning and afternoon observations, with the overall activity of the piglets and gilts higher during the afternoon. Moreover, expected differences in behaviour between trial 1 and trial 2 associated with season of observation were seen. For example, as shown in Table III, a higher frequency of eating behaviour was observed in trial 1 compared to trial 2. This could be related to the fact that trial 1 was carried out during the autumn-winter and, therefore, a higher eating behaviour was performed during the observation period, whereas in trial 2 (spring-summer observations), the eating behaviour was more concentrated at dawn and dusk (i.e. not included in the observation period).

3.2. Performance

No significant differences were observed between the live weights and average daily gains of Nn and NN or Pi NN and Pi nn sired piglets or gilts in any of the trials (Tab. IV). Although the halothane gene has been traditionally associated with an antagonistic relationship between carcass quality and meat quality [14] and it has been said that

Table IV. Mean live weights (s.e.) and average daily gains of the animals observed at the farrowing and fattening pens in trials 1 and 2.

	Trial 1[1]			Trial 2		
	Halothane genotype			Terminal sire		
	NN	Nn		Pi NN	Pi nn	
N (suckling piglets)[2]	30	21	RSD	30	29	RSD
Live weight at 1 day (kg)	1.8	1.8	0.3	1.7	1.6	0.3
Live weight at 20 days (kg)	6.7	6.8	1.2	5.6	5.6	0.9
Average daily gain during farrowing period (kg per day) (1–20 days of age)	0.24	0.25	0.04	0.22	0.20	0.04
N (growing gilts)	40	40	RSD	40	40	RSD
Live weight at 180 days (kg)	103.5	102.9	11.6	102.6	104.8	11.7
Average daily gain during fattening period (kg per day) (20–180 days of age)	0.56	0.56	0.06	0.55	0.56	0.06

[1] Due to experimental design, in Trial 1 gilts and piglets were compared taking into account their halothane genotype as the fixed factor, whereas in Trial 2 "terminal sire" was the fixed factor (see Materials and Methods for more details).
[2] Half of the piglets were males and half females for each halothane genotype.

it definitely improves carcass lean content, there are controversial results in the scientific literature in relation to the growth performance of Nn pigs. They have been found to show a higher [10] or lower average daily gain [3] compared to NN pigs. Our results are in agreement with other studies [12], which did not find significant differences in daily gain or total weight between NN and Nn pigs. Pedersen et al. [12] have suggested that the most relevant differences between NN and Nn pigs are encountered in the composition of the carcass (i.e. reduced fat deposition rate but higher growth rate of muscle fibres in Nn pigs), while the daily gain is unaltered. This would be in accordance with the combined results of this study and those on the carcass quality of the present gilts [4], which revealed a higher lean content in Nn pigs compared to NN with no differences in average daily gain.

Differences in behaviour and performance are considered welfare indicators. This study showed a non-significant effect of the halothane genotype on growth and of little magnitude on behaviour when pigs were subjected to a situation with ad libitum feed and water. However, it must be taken into account that when these gilts were subjected to presumably more challenging situations like transportation [4] or a novelty test [5], differences in responsiveness between NN and Nn individuals were observed, with Nn gilts showing higher cortisol increases, heart rate or lower locomotive activity in the novelty test. Therefore, behavioural differences due to genotype may be expressed when animals are exposed to an "induction" type stressor and this should be considered when assessing the welfare of those individuals.

4. CONCLUSIONS

Heterozygous and halothane negative suckling piglets and growing gilts kept under standard commercial conditions did not differ consistently in their behaviour. Moreover,

no significant differences in body weight or average daily gain between NN and Nn piglets and gilts were found. Therefore, under the present commercial conditions, differences in behaviour could be better explained by other environmental factors like time of day or age than the halothane genotype.

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