The Old School, Brewhouse Hill, Wheathampstead,
Hertfordshire AL4 8AN, UK

Animal Welfare 2008, 17: 155-160
ISSN 0962-7286

Influence of olfactory substances on the heart rate and lying behaviour of pigs during transport simulation

B Driessen*†‡, E Peeters§ and R Geers‡

† Katholieke Hogeschool Kempen, Kleinhoefstraat 4, B-2440 Geel, Belgium
‡ Laboratory for Quality Care in Animal Production, Zootechnical Centre, KU Leuven, Bijzondere weg 12, B-3360 Lovenjoel, Belgium
§ Federal Government Service, Department Animal Welfare, Victor Hortaplein 40/10, B-1060 Brussels, Belgium
* Contact for correspondence and requests for reprints: Bert.Driessen@khk.be

Abstract

This study investigated the effect of olfactory substances on the heart rate and lying behaviour of pigs during transport simulation. Five treatments were tested through the application of each substance to pigs' snouts with a paintbrush. These consisted of: 1) control treatment (wiping without product); 2) 2 ml of a synthetic, maternal-like pheromone; 3) 5 ml of a synthetic, maternal-like pheromone; 4) a commercial, non-relevant odour and 5) 2 ml of a placebo (solvent of the synthetic pheromone without active ingredients). In total, 90 pigs took part in this study and each treatment was tested on a group of three pigs with six replicates per treatment. Pigs were vibrated in the vertical direction in a transport simulator with a frequency of 8 Hz and an acceleration of 3 m s^{-2}. Cardiac activity and lying behaviour during vibration were quantified. The effect of vibration was found to be statistically significant, ie causing an increase in heart rate and numbers of ventricular ectopic beats (VEB). Both 2 and 5 ml of synthetic pheromone were generally found to decrease the minimum, mean, and peak heart rate values in comparison with the other treatments (in particular the control and the non-relevant odour group) but only minimum heart rate reached statistical significance. However, the number of VEBs was highest for these two synthetic pheromone groups during vibration. No dose-dependent synthetic pheromone effects were found and there were no differences in the amount of time pigs spent lying. The use of olfactory substances may support pigs' ability to cope with real transport conditions thereby improving their welfare.

Keywords: *animal welfare, heart rate, olfactory substances, pig, stress, vibration*

Introduction

An important phase in animal production is transportation to the slaughterhouse and work continues towards demonstrating the extent to which this is a stressful event for the animals. Vibration during transport, characterised by frequency, acceleration and direction, may lead to muscular fatigue in such animals (Randall *et al* 1995). A transport simulator was used to investigate the effect of vibration on the physiology of growing pigs and it was shown that heart-rate variability and endocrinological reactions were in keeping with those observed during real transport conditions (Perremans 1999).

The use of pharmaceutical sedatives before transport of animals, to avoid stress and mortality, is currently illegal in the European Community. A potentially acceptable alternative, which may improve welfare during transportation, is the use of naturally-occurring olfactory substances or pheromones, or their synthetic forms. Pheromones can be defined as follows: "substances which are secreted to the outside by an individual and received by another individual of the same species, in which they release a specific reaction, for example, a definitive behaviour or a development process" (Karlson & Lüscher 1959; Birch 1974; Barrows 1995). Cowley and Wise (1970) showed a reduction in the activity of 6-day old mice when kept in the presence of soiled sawdust or urine from a lactating female and an increase in activity when kept in the presence of stock sawdust. Barnett (1963) described how a member of a clan of wild rat was set upon after being removed from the group and kept in an empty cage for some time. Death tends to follow after re-introduction as a result of attacks from the orginal clan members. If the animal is housed on some of his original bedding material during his exile, the harassment does not occur on re-introduction. Hence, the olfactory substances demonstrating a beneficial effect within this experimental design may be defined as pheromones when specificity of species can be proved. Sexual pheromones such as 5α-androst-16-en-3-one reduced agonistic behaviour during the regrouping of pigs (McGlone *et al* 1987; Petherick & Blackshaw 1987). Similarly, mixtures of fatty acids from the udder region of lactating sows, reduced agonistic behaviour between pigs when applied during mixing at weaning, and improved

weight gain afterwards (Pageat & Tessier 1998; Driessen *et al* 2002; McGlone & Anderson 2002). These beneficial effects of olfactory substances were demonstrated for pigs at weaning. Therefore, the main objective of this study was to evaluate if synthetic olfactory substances could reduce stress in growing pigs, during a vibration treatment which simulates transportation, by measuring parameters of heart rate (HR) and lying behaviour.

Materials and methods

Animals and housing

Pigs, weighing approximately 7 kg and heterozygous for the halothane gene, were purchased and kept at the Zootechnical Centre of Lovenjoel (KU Leuven, Belgium) within standardised housing conditions (2.05 ×1.70 m; length × width) in groups of twelve. The animals were given *ad libitum* water and feed. One day prior to commencement of transport simulation, three pigs of approximate weight 22 kg (mean 22.2 [± 4.2] kg) were chosen randomly from different pens and transferred by trolley to a temperature-controlled room to attach devices for measurements of heart rate. It is our assumption that pigs of this bodyweight (as well as being relatively easily handled) will demonstrate physiological stress responses comparable to those of market pigs (Dantzer & Mormède 1983). Pigs were treated and vibrated in groups of three to avoid any potential effects of isolation stress. Five treatments were tested; each on six groups of three pigs, meaning in total 90 pigs were involved in this study.

Treatments

The following five treatments underwent testing: 1) control treatment; 2) 2 ml of a synthetic pheromone; 3) 5 ml of a synthetic pheromone; 4) a commercial non-relevant odour and 5) 2 ml of a placebo. Treatment was administered with a paintbrush onto the snout of pigs immediately prior to the start of vibration. The synthetic pheromone (2) and (3) was a micro-emulsion containing 20% active ingredients, being a mixture of six components (oleic acid, linoleic acid, palmitic acid, myristic acid, lauric acid, and capric acid in a 10/10/7/2/2/1 molar ratio [Suilence®, Ceva, Libourne Cedex, France]), whereas the placebo (5) was the same micro-emulsion but without the active ingredients. These fatty acids were found in the skin secretions of lactating sows (Pageat & Tessier 1998). The control treatment (1) consisted of wiping the snout of the pigs with a dry paintbrush and, for the odour, (4) a commercial spray (hartshorn oil, obtained as an intermediate product of the dry distillation of offal oil for the preparation of animal charcoal [PBH-spray, Intervet, Mechelen, Belgium]), sprayed on to a paintbrush, was used. This product is used commercially to inhibit feather pecking in poultry and ear and tail biting in pigs and was included to help establish whether any differences in response to the pheromone product were due to the product itself or simply a response to the application of an unfamiliar odour. Each vibration session included an identical treatment for each animal. The order of treatments was carried out randomly. After each session the crate was cleaned properly with a detergent and disinfected to avoid carry-over effects. The room was well ventilated and conditioned at a standard air temperature of 22 (± 2)°C.

Preparation of the animals

Animals were treated in accordance with the regulations of the Council Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes (OJEU 1986). The day before vibration simulation, pigs were weighed and anaesthetised with an intramuscular injection of azaperone at 2 mg kg^{-1} (Stressnil, Janssen Pharmaceutica, Beerse, Belgium) and an intraperitonal injection of metomidate at 10 mg kg^{-1} (Hypnodil, Janssen Pharmaceutica, Beerse, Belgium). During anaesthesia, an ambulatory Holter device (Model 43400B Analyzer, Philips Medical System, Eindhoven, The Netherlands) was attached to the pigs in order to record the electrocardiogram for a period of 24 h. Five adhesive leads (Unilect, Maersk Medical, Redditch, UK) were placed, respectively, at the fourth intercostal space right and left of the sternum border and at the fifth intercostal space right and left of the anterior axillary line, with the reference electrode on the fifth rib right of the sternum. Minimum, mean, and peak HR, and the number of ventricular ectopic beats (VEB) were measured (Geers *et al* 1990). Minimum, mean, and peak HR were calculated as the moving average over 12 beats. To avoid damaging the Holter device, pigs were housed individually in metabolic cages (1.00 × 0.90 × 0.92 m; length × width × height) after anaesthesia, allowing auditory but precluding visual contact with other pigs. The recording for the reference period took place during the night between 2200 and 0600h. All pigs were prepared in identical fashion in order to protect against any procedural influence.

Transport simulation

At 0800h on the day following anaesthesia, the group of three pigs were placed together in the vibration simulator crate (1.22 × 0.77 × 0.64) and given a period of one hour to acclimatise to their new surroundings. At 0900h the engine was switched on without any vibration and the pigs were given a further hour to get used to the sound of the engine noise before application of the treatment to the pigs' snouts and initiation of vibration at 1000h. The transport simulator was designed and assembled by Silsoe Research Institute (UK). Pigs appeared very sensitive to a combination of a frequency of 8 Hz and an acceleration of 3 m s^{-2} (Perremans *et al* 1998). This frequency and acceleration was chosen because it had previously been shown to be the frequency and acceleration measured during transport in a trailer (Villé *et al* 1993; Geers *et al* 1994). Vibration lasted until 1200h and the first and second hour were labelled V1 and V2, respectively. Each animal underwent only one of the five possible treatments to avoid training effects (Geers *et al* 1994).

Behavioural observations

During the two-hour vibration period, pigs were observed with a camera (KP-143, Hitachi, Brussels, Belgium) which was suspended above the crate and connected to a monitor. The time (s) each pig spent lying was recorded and the degree of restlessness exhibited by the individual animals was used as a measure of stress (Lambooij 1988).

https://doi.org/10.1017/S0962728600027664 Published online by Cambridge University Press

Table 1 Least-square means (± SEM) of beats per min of the minimum heart rate per treatment during the reference period and transport simulation.

Treatment/item	Reference†	V1‡	V2§
Number of pigs per treatment group	18	18	18
Control	103 ± 6^{a}	117 ± 6^{x,b}	114 ± 6^{x,a,b}
2 ml synthetic pheromone	99 ± 6	95 ± 6^{y}	92 ± 6^{yz}
5 ml synthetic pheromone	92 ± 6	97 ± 7^{y}	87 ± 6^{y}
5 ml non-relevant odour	102 ± 6	108 ± 6^{xy}	107 ± 6^{xz}
5 ml placebo	100 ± 7	106 ± 7^{xy}	103 ± 7^{xy}

† Reference, average of recorded values under sleeping conditions (between 2200 and 0600h).
‡ V1, first hour of the transport simulation.
§ V2, second hour of the transport simulation.
^{a,b} Within a row, means without a common superscript differ: $P < 0.05$.
^{x,y,z} Within a column, means without a common superscript differ: $P < 0.05$.

Table 2 Least-square means (± SEM) of beats per min of the mean heart rate per treatment during the reference period and transport simulation.

Treatment/item	Reference†	V1‡	V2§
Number of pigs per treatment group	18	18	18
Control	128 ± 3^{a}	146 ± 4^{b,x,y}	142 ± 4^{b,x,y}
2 ml synthetic pheromone	123 ± 4^{a}	134 ± 4^{b,x}	131 ± 5^{a,b,x}
5 ml synthetic pheromone	119 ± 4^{a}	136 ± 4^{b,x}	132 ± 4^{b,x}
5 ml non-relevant odour	130 ± 5^{a}	149 ± 5^{b,y}	148 ± 5^{b,y}
5 ml placebo	128 ± 5^{a}	143 ± 5^{b,x,y}	146 ± 5^{b,y}

† Reference, average of recorded values under sleeping conditions (between 2200 and 0600h).
‡ V1, first hour of the transport simulation.
§ V2, second hour of the transport simulation.
^{a,b} Within a row, means without a common superscript differ: $P < 0.05$.
^{x,y,z} Within a column, means without a common superscript differ: $P < 0.05$.

Statistical analyses

Data were analysed using the Mixed procedure of SAS (SAS Institute Inc, Cary, NC, USA) with 'treatment' and 'period' as main variables and bodyweight and sex as covariates (Peeters *et al* 2005). Group and animal were used as random factor, taking into account the relationship of measurements from the same group and animal, respectively. This way, the individual pig functioned as the experimental unit. No interactions between treatment and period were significant, so this term was omitted in the model. The VEB data were transformed for normality (ln x). Least-square means were calculated and differences between treatments and period were separated using orthogonal contrasts. The means of transformed data were re-transformed, and the standard errors were calculated using the delta method (Serfling 1980).

Results

During V1, the minimum HR of pigs treated with the synthetic pheromone (2 or 5 ml) was lower than the minimum HR of the control pigs ($P = 0.01$ and $P = 0.02$, respectively; Table 1). The next hour, an additional difference was found between pigs wiped with 5 ml of synthetic pheromone and those with the non-relevant odour ($P = 0.03$). An increase in the minimum HR was seen from the reference period to V1 ($P = 0.02$) for the control pigs.

During V1, pigs treated with the non-relevant odour had a higher mean HR than pigs treated with 2 ml and 5 ml of synthetic pheromone ($P = 0.02$ and $P = 0.03$, respectively) (Table 2). During V2, both synthetic pheromone treatments differed from placebo treatment (both $P = 0.03$) as well as non-relevant odour treatment (both $P = 0.01$). For all treatments, the reference mean HR was lower than the mean HR

https://doi.org/10.1017/S0962728600027664 Published online by Cambridge University Press

Table 3 Least-square means (± SEM) of beats per min of the peak heart rate per treatment during the reference period and transport simulation.

Treatment/item	Reference†	V1‡	V2§
Number of pigs per treatment group	18	18	18
Control	181 ± 5^{a}	199 ± 5^{b,x,z}	193 ± 5^{b}
2 ml synthetic pheromone	173 ± 6^{a}	186 ± 6^{b,y,z}	190 ± 6^{b}
5 ml synthetic pheromone	173 ± 5^{a}	194 ± 5^{b,x,y}	189 ± 5^{b}
5 ml non-relevant odour	187 ± 6^{a}	207 ± 6^{b,x}	198 ± 6^{a,b}
5 ml placebo	172 ± 6^{a}	182 ± 6^{a,y}	200 ± 6^{b}

† Reference, average of recorded values under sleeping conditions (between 2200 and 0600h).
‡ V1, first hour of the transport simulation.
§ V2, second hour of the transport simulation.
^{a,b} Within a row, means without a common superscript differ: $P < 0.05$.
^{x,y,z} Within a column, means without a common superscript differ: $P < 0.05$.

Table 4 Least-square means (± SEM) of beats per min of the number of ventricular ectopic beats (VEB) per treatment during the reference period and transport simulation.

Treatment/item	Reference†	V1‡	V2§
Number of pigs per treatment group	18	18	18
Control	3 ± 1^{a,x}	28 ± 9^{b}	18 ± 6^{b,x}
2 ml synthetic pheromone	8 ± 3^{a,y}	67 ± 24^{b}	52 ± 19^{b,y,z}
5 ml synthetic pheromone	8 ± 3^{a,y}	68 ± 24^{b}	78 ± 28^{b,y}
5 ml non-relevant odour	5 ± 2^{a,x,y}	44 ± 16^{b}	25 ± 9^{b,x,z}
5 ml placebo	9 ± 3^{a,y}	59 ± 22^{b}	65 ± 24^{b,y,z}

† Reference, average of recorded values under sleeping conditions (between 2200 and 0600h).
‡ V1, first hour of the transport simulation.
§ V2, second hour of the transport simulation.
^{a,b} Within a row, means without a common superscript differ: $P < 0.05$.
^{x,y,z} Within a column, means without a common superscript differ: $P < 0.05$.

during V1 and V2 ($P \leq 0.01$), with exception of the difference between the reference values and V2 for the 2 ml of synthetic pheromone treatment.

Peak HR results are shown in Table 3. The peak HR increased for most treatments during V1 and V2 when compared with the reference period. Only during V1 were differences between the different treatments found: animals treated with the placebo had the lowest peak HR, which differed from the peak HR of the control pigs ($P = 0.02$) and the pigs treated with the non-relevant odour ($P = 0.002$). The highest peak HR was observed after treatment with the non-relevant odour, differing with the 2 ml of synthetic pheromone treatment ($P = 0.01$).

Within a treatment, the probabilities for equal means of number of VEB between periods of measurements were lower than 0.0001 (Table 4). During the reference period and V2, control pigs showed a lower number of VEB compared to pigs treated with 2 ml of synthetic pheromone ($P = 0.02$ and $P = 0.04$, respectively), 5 ml of synthetic pheromone ($P = 0.02$ and $P = 0.004$, respectively), and the placebo ($P = 0.001$ and $P = 0.001$, respectively). In the last hour of the vibration simulation, an additional difference between the treatment with 5 ml of synthetic pheromone and the non-relevant odour was found ($P = 0.03$).

No differences in lying duration between treatments were found in the first and second hour of vibration (Table 5). The time spent lying during V2 was higher than during V1 for the control pigs ($P = 0.01$) and the pigs treated with the non-relevant odour ($P = 0.001$) and the placebo ($P = 0.0002$).

Discussion

Heart-rate measurements and behaviour are considered to be appropriate indicators of stress and welfare status in pigs (van Putten & Elshof 1978; Mayes & Jesse 1988; Lambooij & van Putten 1993). Pigs exposed to either 2 or 5 ml of the synthetic pheromone appeared to cope better with the vibration stress as the minimum HR was significantly lower

https://doi.org/10.1017/S0962728600027664 Published online by Cambridge University Press

Table 5 Least-square means (± SEM) of time (in min/s) pigs spent lying per treatment during the reference period and transport simulation.

Treatment/item	V1[‡]	V2[§]
Number of pigs per treatment group	18	18
Control	35 min 58 s ± 4 min 6 s[a]	39 min 13 s ± 2 min 46 s[b]
2 ml synthetic pheromone	35 min 20 s ± 3 min 3 s	38 min 16 s ± 2 min 48 s
5 ml synthetic pheromone	40 min 57 s ± 2 min 49 s	39 min 24 s ± 2 min 35 s
5 ml non-relevant odour	40 min 18 s ± 3 min 16 s[a]	48 min 23 s ± 3 min[b]
5 ml placebo	31 min 57 s ± 3 min 26 s[a]	41 min 44 s ± 3 min 9 s[b]

[‡] V1, first hour of the transport simulation.
[§] V2, second hour of the transport simulation.
[a,b] Within a row, means without a common superscript differ: $P < 0.05$.

than that of the control group, during vibration. The minimum HR was even lower during the second hour of the transport simulation than it was during the reference period (under sleeping conditions) for the pigs treated with the synthetic pheromone. These effects may be due, in part, to the release of endogenous opioids in response to the stressor (Morris *et al* 1990) and a better ability to cope with the new environment. It also suggests that the synthetic pheromone may have beneficial effects; similar to those reported for other substances (Pageat & Tessier 1998). The mean HR reflects the general response of the pigs to the vibration treatment and gives an indication of animals' welfare and comfort (Perremans *et al* 1997). Only the mean HR of pigs treated with 2 ml of synthetic pheromone returned to the range of resting levels during the second hour of the transport simulation. Measurement of this HR variable revealed a greater reduction in stress in these pigs with application of the synthetic pheromone compared with the non-relevant odour (during both hours of vibration) and the placebo (during the second hour of vibration). Lower values for the peak HR were found after treatment with the placebo, compared with both the control and the non-relevant odour treatment, but, overall, better results were obtained with the synthetic pheromone compared with the placebo, which demonstrates the effect of the active ingredients. Pigs treated with the commercial non-relevant spray had high values, so it may be concluded that the synthetic pheromone, itself, was effective at lowering stress during transport simulation and that the pigs were responding to properties within the product and not simply to the application of a non-relevant odour. The heart muscle is more excitable during acute stress, resulting in a higher degree of irregular heart beats or VEB that are not generated in the pacemaker of the heart, but elsewhere in the myocardium (Ellestad 1987). Therefore, VEB can be thought of as being more independent from body movement than HR. During the first hour the number of VEB was higher during vibration. In the second hour of the vibration, pigs treated with the placebo or the synthetic pheromone (2 or 5 ml) showed a higher number of VEB than the control group. However, these differences were already shown to be significant during the reference period, therefore individual differences between pigs may simply be due to the the pigs' natural heart function.

There were no differences between treatments with respect to lying behaviour during the first and second hour of vibration. It is worth noting the lack of a significant increase in time spent lying by pigs after treatment with the synthetic pheromone (2 or 5 ml) from the first to the second hour of vibration. This is in contrast to the other treatments and, as such, relaxing properties may not be attributable to the synthetic pheromone. Similarly, Andersen *et al* (2000) found that a similar product induced more standing and walking in weaned pigs. Thus, the improvement to welfare during transport through influencing pigs' behaviour after application of the synthetic pheromone is more likely to be due to a reduction in aggressive interactions between pigs, as has been shown in related studies with these olfactory substances (Pageat & Tessier 1998; McGlone & Anderson 2002).

According to current thinking, olfactory substances and pheromones act through the central nervous system to alter behaviour (Petherick & Blackshaw 1987; Tirindelli *et al* 1998). The synthetic pheromone administered to pigs in this experiment was seen to alter their electrocardiogram. It may be that the product applied affects the functioning of the pigs' central nervous system by acting through the vomeronasal organ and changing the set points for stress perception. The improvement to welfare has still to be investigated.

In summary, an HR-lowering effect of the synthetic pheromone, originally isolated from skin secretions of lactating sows, was observed without any effect on the time pigs spent lying during the vibration. For all variables observed, a clearly visible dose effect relationship for the synthetic pheromone was not found.

Animal welfare implications

The use of beneficial olfactory substances would be an advantage in real transport conditions from both an animal's and a consumer's point of view. Firstly, treatments can be considered as organic due to the substance's natural origin and mode of action. Secondly, from a practical perspective, they are easily applied.

https://doi.org/10.1017/S0962728600027664 Published online by Cambridge University Press

Acknowledgements

Funding was co-provided by a project (MML-contract S-5913) by the Ministry of Agriculture and Small Enterprises of Belgium. Dr S Perremans supported the experimental work, and Professor G Verbeke (Biostatistical Centre, KU Leuven) advised on the statistical analysis.

References

Andersen IL, Andenaes H, Boe KE, Jensen P and Bakken M 2000 The effects of weight asymmetry and resource distribution on aggression in groups of unacquainted pigs. *Applied Animal Behaviour Science 68*: 107-120

Barnett SA 1963 *A Study in Behaviour*. Methuen & Co: London, UK

Barrows EM 1995 *Animal Behavior Desk Reference*. CRC Press Inc: Boca Raton, Florida, USA

Birch MC 1974 Introduction. In: *Pheromones* pp 1-7. North-Holland Publishing Company: Amsterdam, The Netherlands

Cowley JJ and Wise DR 1970 Pheromones, Growth and Behaviour. In: Porter R and Birch J (eds) *Chemical Influences on Behaviour* pp 144-170. CIBA Foundation Study Group: Churchill, London, UK

Dantzer R and Mormède P 1983 Stress in farm-animals - a need for re-evaluation. *Journal of Animal Science 57*: 6-18

Driessen B, Peeters E, van de Water G and Geers R 2002 Influence of olfactory substances on growth performance, carcass damage and meat quality of pigs. In: van der Honing Y (ed) *Book of Abstracts of the 53rd Annual Meeting of the European Association for Animal Production* pp 150. 1-4 September 2002, Cairo, Egypt: Wageningen Academic Publishers: Wageningen, The Netherlands

Ellestad MH 1987 *Stress Testing: Principles and Practice*, *Third Edition*. FA Davis Company: Philadelphia, PA, USA

Geers R, Bleus E, van Schie T, Villé H, Gerard H, Janssens S, Nackaerts G, Decuypere E and Jourquin J 1994 Transport of pigs different with respect to the halothane gene: stress assessment. *Journal of Animal Science 72*: 2552-2558

Geers R, Parduyns G, Goedseels V, Bosschaerts L and De Ley J 1990 Skeletal muscularity and heart function in growing piglets. *Annales de Recherches Veterinaires 21*: 231-236

Karlson P and Lüscher M 1959 'Pheromones': a new term for a class of biologically active substances. *Nature 183*: 55-56

Lambooij E 1988 Road transport of pigs over a long distance: Some aspects of behaviour, temperature and humidity during transport and some effect of the last two factors. *Animal Production 46*: 257-263

Lambooij E and van Putten G 1993 Transport of pigs. In: Grandin T (ed) *Livestock Handling and Transport* pp 213-231. CABI: Wallingford, UK

Mayes H and Jesse G 1988 Activity effects on the heart rate of feeder pigs. *Applied Engineering in Agriculture 4*: 156-158

McGlone JJ and Anderson DL 2002 Synthetic maternal pheromone stimulates feeding behavior and weight gain in weaned pigs. *Journal of Animal Science 80*: 3179-3183

McGlone JJ, Curtis SE and Banks EM 1987 Evidence for aggression modulating pheromones in prepuberal pigs. *Behavioral and Neural Biology 47*: 27-39

Morris M, Salmon P, Steinberg H, Sykers EA, Bouloux P, Newbould E, McLoughlin L, Bessers GM and Grossman A 1990 Endogenous opioids modulate the cardiovascular response to mental stress. *Psychoneuroendocrinology 15*: 185-192

OJEU 1986 Council Directive 86/64/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. *Official Journal of the European Union L 358*: 0001-0028

Pageat P and Teissier Y 1998 Can a pig pheromone analogue improve daily weight gain and food conversion efficiency post-weaning? *Proceedings of the 15th IPVS Congress* pp 413. 5-9 July 1998, Birmingham, UK: University Press: Nottingham, UK

Peeters E, Neyt A, Beckers F, de Smet S, Aubert AE and Geers R 2005 Influence of supplemental magnesium, tryptophan, vitamin C, and vitamin E on stress responses of pigs to vibration. *Journal of Animal Science 83*: 1568-1580

Perremans S 1999 *Physiological and ethological responses of pigs during simulation of transport. Acta Biomedica Lovaniensia No. 201*. University Press: Leuven, Belgium

Perremans S, Randall J, Rombouts G, Duchateau W, Villé H and Geers R 1997 Welfare monitoring of pigs in relation to transport. In: Bottcher RW and Hoff SJ (eds) *Proceedings of the Fifth International Symposium on Livestock Environment* pp 138-145. 29-31 May 1997, Bloomington, Minnesota. ASAE: Michigan, USA

Perremans S, Randall JM, Allegaert L, Stiles MA, Rombouts G and Geers R 1998 Influence of vertical vibration on heart rate of pigs. *Journal of Animal Science 76*: 416-420

Petherick JC and Blackshaw JK 1987 A review of the factors influencing the aggressive and agonistic behaviour of the domestic pig. *Australian Journal of Experimental Agriculture 27*: 605-611

Randall JM, Stiles MA, Geers R, Schütte A, Christensen L and Bradshaw RH 1995 Transport of pigs: vibration and discomfort. In: *Proceedings of the United Kingdom Informal Group Meeting on Human Response to Vibration*. 18-20 September 1995, Silsoe, UK. Silsoe Research Institute: Silsoe, Bedford, UK

Serfling RJ 1980 Transformations of Given Statistics. In: *Approximation Theorems of Mathematical Statistics, First Edition* pp 117-137. John Wiley and Sons: New York, USA

Tirindelli R, Mucignat-Caretta C and Ryba NJP 1998 Molecular aspects of pheromonal communication via the vomeronasal organ of mammals. *Trends in Neurosciences 11*: 482-486

van Putten G and Elshof WJ 1978 Observations on the effect of transport on the well being and lean quality of slaughter pigs. *Animal Regulations Studies 1*: 247-271

Villé H, Bertels S, Geers R, Janssens S, Goedseels V, Parduyns G, van Bael J, Goossens K, Bosschaerts L, de Ley J and Heylen L 1993 Electrocardiogram parameters of pigs during housing, handling, and transport. *Animal Production 56*: 211-216

https://doi.org/10.1017/S0962728600027664 Published online by Cambridge University Press