Fear assessment in pigs exposed to a novel object test.

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Abstract

The experiment aimed to study approach and locomotive behaviour as indicators of fear in a novel object test carried out in pigs. Thirty post-weaning (30 kg) and 30 finishing (90 kg) pigs were exposed to visual, auditory and olfactory novel stimuli during 2 different experiments. The facilities consisted of a test pen in which a trough was located. The trough contained chopped apples. Once the animals were trained to enter the test pen individually they were subjected to 3 different fear stimuli. These stimuli were applied in the test pen and next to the trough. The variables studied were feeding behaviour, approach behaviour (the distance and position of the animal with respect to the trough) and locomotive behaviour (general activity, reluctance to move, turning back and retreat attempts). Two groups were studied: saline and midazolam treated group. Twenty minutes before the start of the sessions, 15 post-weaning and finishing pigs received an intramuscular injection of 0.20 mg/kg and 0.15 mg/kg, respectively, midazolam (Dormicum®). The saline pigs (15 animals per group) were injected with saline. The administration of midazolam increased the feeding behaviour and approaching behaviour, and reduced the locomotive behaviour. In front of the visual and

olfactory stimuli post-weaning pigs showed a higher general activity than finishing pigs, but the contrary was found when the auditory stimulus was applied. The olfactory stimulus was more related to the turning back behaviour, whereas the visual stimulus was more related to retreat attempts. Although it could be concluded that reluctant to move was the most common response to the different fear stimuli applied in our study regardless of the age of animals, the combination of reluctant to move and turning back would be a good criterion to assess fear in domestic pigs. The use of midazolam as anxiolytic for studies of fear in commercial conditions in pigs is recommended.

Keywords: auditory stimulus, behaviour, fear, midazolam, novel object test, olfactory stimulus, pigs, visual stimulus.

Introduction

Fear and anxiety are 2 emotional states induced by the perception of a danger or a potential danger, respectively, that threaten the integrity of the animal (Boissy, 1995). Fear and anxiety involve physiological and behavioural changes that prepare the animal to cope with the danger (Forkman et al., 2007). From an ecological point of view, suddenness, unfamiliarity and unpredictability are the key features of a predatory attack (Forkman et al., 2007), so these factors should be considered when the responsiveness to a stimulus is studied in pigs. The fear response depends also on the type of the threat and decreases with the age of the animal (Forkman et al., 2007). The behavioural pattern that can show pigs can be grouped in active defence (attack, threat), active avoidance (flight, hiding, escape) and passive avoidance (immobility; Erhard et al., 1999). However, because of the complexity of the mechanisms underlying fear-related responses, a given behaviour can not be attributed to any single emotion (Boissy, 1998).

Therefore, a combination of different types of behaviour should be used to assess fear. The most common fear tests are open field, novel object and approach tests (Forkman et al., 2007).

Sudden or unfamiliar events are often applied to assess the animal's fearfulness (Boissy, 1995). Unfamiliarity is applied in the open field and in the novel object test (Forkman et al., 2007). In the open field test, the animal is placed in a novel area and the response to the new situation is assessed by means of general activity, escape attempts, reluctant to move, eliminate behaviour, and vocalisations (Beattie et al., 1995; Forkman et al., 2007; Von Borrell and Ladewig, 1992). In the novel object test, the unfamiliarity is induced by the presence of a new stimulus. The fear or interest towards the novel stimulus is measured by means of the latency, frequency and duration of contact, exploration and attention without physical contact (Forkman et al., 2007). The novel stimuli are usually visual, although it can be also concern olfactory (Jones et al., 2000) and auditory cues (Anderson et al., 1999; Hutson et al., 2000). Tests for unfamiliarity could be assessed with the presentation of an aversive odour stimulus such as a high concentration of CO_2 (Velarde et al., 2007; Raj and Gregory, 1995). Suddenness can be induced by the presentation of a ball falling suddenly from the ceiling in front of the animal (Romeyer and Bouissou, 1992) or just appearing in front of the animal when a through is opened. Finally, the sound of a horn around the animal at different times could introduce an unpredictability effect to the suddenness and unfamiliarity of the stimulus. In some studies combined tests are carried out, for example by letting a novel object after an habituation period within the test arena (Forkman et al., 2007). In this case, a combination of parameters assessed in both type of tests can be used. The present study aims to identify some behavioural indicators that could be measure in practical conditions fear in pigs on farm, during transport (loading and unloading) and at the

slaughterhouse. In these conditions, animals are subjected to new environments and novel stimuli in a sudden, unfamiliarity and unpredictability way. Therefore, in the present study a combination of the open field and novel object tests was carried out. In consequence, most of the behavioural parameters used have been assessed in several studies of fear, in open field or novel object tests (Hemsworth, 2000, Erhard et al., 1999; Beattie et al., 1995; Boissy, 1995; von Borell and Ladewig, 1992).

Some studies use also pharmacological validation, revealing sensitivity of allegedly fear-related behaviours to treatment with angiogenic and anxiolytic drugs (Choleris et al., 2001; Rex et al., 1996; Sánchez, 1995). In pigs, Dantzer (1977), Arnone and Dantzer (1980) and Andersen et al., (2000) used diazepam (Valium®), to compare the behaviour in an elevated plus-maze of animals non treated and treated with the anxiolytic. However, the use of other benzodiazepines is also possible. In the present study the treated pigs received a short acting water soluble imidazobenzodiazepine, (Midazolam, Dormicum®), with an anxiolitic effect similar to diazepam but with a quick onset and, due to a rapid metabolic inactivation, a much shorter duration of action (Pieri, 1983).

The objective of the present study was to assess the fear behaviour of post-weaning and finishing pigs exposed to a visual, auditory and olfactory stimulus. The hypothesis to be tested was that fear and anxiety can be expressed by means of certain types of approaching and locomotive behaviour. Assuming that anxiolytic agents as midazolam reduce fear, this avoidance behaviour would be higher among the pigs not treated with an anxiolytic agent compared to those treated with the agent.

Materials and Methods

Animals and housing conditions

Thirty post-weaning (body weight 30.4 ± 0.69 kg) and 30 finishing pigs (body weight 91.1 ± 1.8 kg) were used in 2 different trials. Pigs were bred under commercial conditions, and 3 days before the start of the experiment transported to the experimental facilities. The pigs were grouped into 15 pairs and housed in pens of 90 x 180 cm with water and food available *ad libitum*.

Test facilities

The test pen consisted of a 16 m^2 pen separated from the housing pen through a corridor with a door on each side (Figure 1). The corridor was 3.5-4.0 m in length, 1.2 m wide and bordered by steel panels. Pigs entered and exited the test pen through the same door. In the wall opposite the door a trough was installed with a lid linked to a rope to be closed and opened manually by an operator. A curtain was placed between the trough and the operator to avoid the animals being distracted during the experiment. The trough contained 3-4 kg of chopped apple. The floor of the test pen was marked with a white line every 40 cm, starting at 20 cm away from the trough. These lines divided the pen into 7 areas. Pigs entered and exited the test pen through the same door. Two video cameras were placed above the trough and on the door. They were connected to a video recorder and a television located behind the curtain. The first camera was placed above the trough to record the feeding behaviour and the second one on the door to record the rest of parameters assessed.

Test procedure

Before the start of each experiment, all animals were weighted and randomly divided into 3 groups of 5 pairs (blue, green and red; Figure 1). In each group, pigs were individually marked with a number from 1 to 10. Animals from the same pair were

identified with consecutive numbers. Each experiment lasted 25 days and except during the resting days, pigs were individually and in the same order (from 1 to 10) subjected to 1 session per day (from 12.00 to 15.00h) according to the protocol of Table 1. During the 2 first sessions, pigs were trained to enter the test pen and eat the chopped apples of the trough. After 2.5 min, the pen door was opened and the animals were allowed to return to the housing pen. During the control and treatment sessions, the trough remained closed when the pig entered the test pen, and after 30 seconds the trough was opened. Two minutes later, the door was opened to let the animal return to the housing pen. The trough was closed after the animal left the test pen and before the next one entered. During the treatment sessions, the animals were exposed to a novel stimulus when the trough was open. Three different types of novel stimuli were used. 1) As a visual stimulus, a ball hung inside the trough. When the lid was opened the ball was elevated 15-20 cm above the trough, and maintained in this position until the animal left the test pen. Then, the ball was returned into the trough and closed. 2) As an auditory stimulus, a horn located behind the curtain and next to the trough. It was sounded twice, when the through was opened and 1 minute later. 3) As an olfactory stimulus, the trough filled with 90% CO_2. The concentration of CO_2 was monitored with a mobile infrared sensor (Checkpoint O_2/CO_2, PBI Dansensor A/S, Denmark). Each stimulus was applied to each animal once (Table 1). Twenty minutes before the start of the treatment session, 1 pig of the pair housed together (midazolam pig) received an intramuscular injection of 0.20 mg/kg midazolam (Dormicum®, Roche; 50mg/10 ml) in the first experiment (post-weaning pigs), and of 0.15 mg/kg midazolam in the second experiment (finishing pigs). The other pig (saline pig) received an intramuscular injection with an equivalent volume of saline (Phisiologic Braun, B. Braun Medical, S.A.).

151 Measurements

152 In the test pen, the feeding behaviour, approaching behaviour and locomotive behaviour
153 were scored for 2 minutes, from when the trough was opened until the door of the test
154 pen was opened (Table 2).

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156 Statistical analysis

157 As the data obtained were not normally distributed, the statistical analysis was carried
158 out with non-parametric tests using the Statistical Analysis System (SAS; software SAS
159 Institute Inc., Cary, NC; 1999-2001). The percentage of time spent for pigs on the
160 trough and distant area or in a head, back and side position, the general activity, and the
161 number of reluctant to move, turning back and retreat attempts were analysed by the
162 Proc GENMOD with Poisson or negative binomial distribution (Cameron and Trivedi,
163 1998). The residual maximum likelihood was used as a method of estimation. The least
164 square means of fixed effects (LSMEANS) were compared when the analysis of
165 variance indicated significant differences. The presence of feeding behaviour was also
166 analysed using Proc GENMOD with a binomial distribution. The fixed effects included
167 in the models were session (treatment and control), treatment (midazolam and saline),
168 stimulus (visual, auditory and olfactory), age (post-weaning and finishing) and their
169 interactions. The significance level was fixed at $P < 0.05$.

170

171 **Results**

172 Table 3 shows the measures of midazolam and saline treated pigs during control and
173 treatment sessions. Both midazolam post-weaning and finishing pigs increased the
174 feeding behaviour, the time on the trough area and the time in a head position. However,
175 they decreased the time on the distant area, time in back position, the general activity

and incidence of reluctance to move during treatment sessions in comparison to control sessions ($P < 0.05$, in all cases). On the other hand, both saline post-weaning and finishing pigs decreased the time on the trough area and time in a back position, and increased the incidence of retreat attempts during treatment sessions in comparison to control sessions ($P < 0.05$, in all cases, Table 3).

During treatment sessions, midazolam post-weaning pigs increased the feeding behaviour, the time spent on the trough area and the time in a head position and decreased the time spent on the distant area and in the incidence of reluctant to move, turning back and retreat attempts when compared with saline post-weaning pigs ($P< 0.05$ in all cases, Table 4). In finishing pigs, the reluctance to move was lower in midazolam than in saline pigs ($P < 0.05$, Table 4).

In post-weaning pigs, differences were found between stimuli applied on time on the trough area, general activity, incidence of turning back and retreat attempts in saline treated animals and on time on the distant area, time in a back and side position and incidence of turning back and retreat attempts in midazolam pigs ($P < 0.05$ in all cases; Table 4). In finishing pigs, differences were found among stimuli applied on time on the distant area, time in a head and side position and incidence of turning back and retreat attempts in saline treated animals and on the incidence of retreat attempts in midazolam treated pigs ($P < 0.05$ in all cases, Table 4).

No significant differences were found between post-weaning and finishing pigs on the feeding behaviour, time on the trough or on the distant area, general activity and reluctant to move for the 3 different fear stimuli applied in both midazolam and saline

pigs. However, midazolam treated finishing pigs spent more time in side position than midazolam treated post-weaning pigs when the auditory and the olfactory stimuli were applied ($P < 0.01$). On the other hand, midazolam treated finishing pigs spent more time in the back position than midazolam treated post-weaning pigs when olfactory stimulus was applied and saline finishing pigs spent less time in the back position than saline post-weaning pigs when the visual stimulus was applied ($P < 0.05$ in both cases). Finishing pigs showed more turning back movements than post-weaning pigs in both treatments (midazolam and saline) when the auditory stimulus was applied and in the midazolam treated animals when the visual stimulus was applied ($P < 0.05$, in all cases). Finally, midazolam treated finishing pigs showed more retreat attempts than midazolam treated post-weaning pigs when the visual stimulus was applied ($P < 0.05$).

Discussion

Pharmacological effect

The administration of midazolam in both post-weaning and finishing pigs reduced the avoidance distance to the trough, the general activity and the number of reluctant to move, turning back and retreat attempts when a fear stimulus was applied. In addition, midazolam post-weaning pigs increased the feeding behaviour. The acute administration of midazolam could increase pig appetite, as described by Yerbury and Cooper (1987) in rats and De Jong (1987) in ruminants. However, this feeding increase is related to an increase in the meal size rather than an increase in the meal frequency (Cooper and Yerbury, 1986; De Jong, 1987). In consequence, the administration of midazolam is not likely to increase the number of animals that ate apples. In contrast, the anxyolitic effects of midazolam reduced the fear response of pigs, as it was also described by Andersen et al. (2000) for diazepam in an elevated plus-maze. Although to our

knowledge, midazolam has not previously been used to assess fear in pigs, we carried out preliminary studies to determine the dosage of midazolam that induces anxiolytic effect without impairing locomotion (Andersen et al., 2000). In this previous study, it was found that animals which were administered with 0.15-0.20 mg/kg i.m midazolam showed less avoidance behaviour (measured as distance to a novel stimulus) than animals with lower dosages or control animals (saline treated, pers. obs.). In addition, it was also observed that those animals did not show the locomotive discoordination (measured as difficulty to walk) observed in animals treated with higher dosages of midazolam (0.4, 0.6 and 0.8 mg/kg). Therefore, according to the results obtained in the present study, midazolam dosages of 0.20 mg/kg for animals around 30 kg and 0.15 mg/kg for animals around 100 kg body weight could reduce the fear-related responses in pigs.

Stimulus effect in post-weaning pigs

The visual and olfactory stimuli were associated to an area, the trough. The ball appeared about 15-20 cm above the trough and high concentration of CO_2 was contained inside it. On the other hand, the horn sounded just in the surroundings and the pigs could not associate the auditory stimulus with the trough. Therefore, when the fear stimuli was localized (in the case of the visual and olfactory stimuli), post-weaning pigs were excited (with an increase in general activity), and when the stimulus was not localized, pigs were quiet. Futhermore, when the ball appeared, pigs remained in side or back position more time, avoiding visual contact with the trough, than when olfactory or auditory stimuli were applied.

Stimulus effect in finishing pigs

Saline finishing pigs remained more time in back position with the olfactory or auditory stimuli than with the visual one. In fact, when the fear stimuli were localized, finishing pigs were quieter, and when an unpredictable stimulus without localisation was applied the pigs were more excited (with an increase in general activity and turning back movements). Furthermore, retreat attempts was not significantly different between midazolam and saline finishing pigs when the auditory and visual stimuli were applied, and therefore not valid to assess fear when these two types of stimuli are applied.

Age effect

When the visual stimulus was applied, both midazolam and saline post-weaning pigs remained in back or side position more time than saline and midazolam finishing pigs, the last staying in a head position. The different behaviour between ages could be due to a combination of factors. The first cause might be that the size of the visual stimulus (the ball) could be big enough to induce fear in post-weaning pigs but not in finishing pigs. As the ability to cope with an event can change the emotional experience of the animal towards the situation (Forkman et al., 2007), for finishing pigs the fearfulness of the same ball could be lower than for post-weaning pigs. The second hypothesis is that post-weaning and finishing pigs show a different behavioural pattern to the same fear stimulus, with differences in the active and passive avoidance (Erhard et al., 1999). The third hypothesis is that post-weaning pigs are more fearful or react more extremely than finishing pigs to the same stimulus. In accordance with this last hypothesis, Janczak et al. (2003) observed that in a novel object test, investigation behaviour increased in pigs aged 24 weeks compared with 8 weeks, and concluded that fear and anxiety decrease with age. Some authors argued that behaviour controlled by simple stimulus-response associations in young animals may later, in older ones, come under the control of more

276 discriminative cognitive processes (Toates, 2000 and Janczak et al., 2003), becoming, in
277 these older animals, a more stimulus-specific response. In this case, the more
278 exaggerated response of post-weaning pigs to the visual stimulus in comparison to
279 finishing pigs could have occurred because whereas the finishing pigs were analysing
280 the potential danger of the stimulus (Toates, 2000), post-weaning pigs were just
281 avoiding it. In consequence, the unpredictability of a stimulus could increase the
282 excitability of finishing pigs but not in post-weaning pigs.
283
284 **Validity**
285 Any behaviour measured to assess general fear in pigs needs to be consistent among
286 ages and types of fear stimuli. Although in this study feeding behaviour, distances and
287 position relative to the trough and general activity were considered, these parameters are
288 not valid during loading and unloading at the slaughterhouse, because in most cases, the
289 assessment is carried out with the animals in movement and no evidence of the novel
290 "fear" stimuli exists. In contrast, types of behaviour such as reluctant to move, turning
291 back and retreat attempts could be appropriate parameters to measure fear in such
292 conditions. In this context, reluctant to move, defined by Erhard et al. (1999) as a
293 passive avoidance behaviour, could be the most valid measure, because no differences
294 were found between ages or fear stimuli applied, but a clear treatment effect
295 (midazolam *vs* saline). Furthermore, it was the most frequent response in pigs exposed
296 to any of the stimuli. On the other hand, turning back behaviour is associated with
297 active avoidance (Erhard et al., 1999) and in the present study, in finishing pigs,
298 increased at the same time increased the general activity, a common indicator of fear in
299 a novel object test (Von Borell and Ladewig, 1992; Beattie et al., 1995). However,

300 turning back behaviour could be not a valid measure in finishing pigs when a visual
301 stimulus is applied.
302 On the other hand, due to the complexity of the mechanisms underlying fear-related
303 responses, it is not possible to attribute a single behaviour to an emotion such as fear
304 (Boissy, 1998). Therefore, although a passive avoidance (reluctant to move) was the
305 most common response shown in pigs to the different fear stimuli applied regardless of
306 the age of animals, the combination of reluctant to move (as passive avoidance
307 indicator) and turning back (as active avoidance behaviour) would be a valid tool to
308 assess fear in domestic pigs after the study of its feasibility and repeatability in practical
309 conditions.
310

311 **Conclusion**

312 Midazolam has anxiolitic effects in post-weaning and finishing pigs exposed to novel
313 stimuli. Although a study about their reliability is needed, reluctant to move and turning
314 back are valid measures of fear in pigs. However, only reluctant to move showed no
315 differences between ages and stimuli. .
316

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399 **Table 1.** Experimental protocol for experiments 1 and 2.

Day	Green Group	Red Group	Blue Group
1 and 2	Training	Training	Training
3 and 4	Control	Control	Control
5 and 6	Resting	Resting	Resting
7 and 8	Control	Control	Control
9	Visual	Control	Control
10	Control	Auditory	Control
11	Control	Control	Olfactory
12 and 13	Resting	Resting	Resting
14 and 15	Control	Control	Control
16	Control	Visual	Control
17	Control	Control	Auditory
18	Olfactory	Control	Control
19 and 20	Resting	Resting	Resting
21 and 22	Control	Control	Control
23	Control	Control	Visual
24	Auditory	Control	No session
25	No session	Olfactory	No session

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415 **Table 2.** Definition of behavioural measures recorded in post-weaning and finishing
416 pigs during the application of auditory, olfactory and visual stimuli in the test pen.

Measure[1]	Definition
Feeding Behaviour	Pigs eating the apples of the trough
Approaching Behaviour	
Time spent in the trough area	Percentage of time spent for pigs in the closest area to the trough (<20cm) in relation to the total time of the test and according to the white lines on the floor.
Time spent in the distant area	Percentage of time spent for pigs in the closest area to the door (more than 220 cm away from the trough) in relation to the total time of the test and according to the white lines on the floor.
Time spent in head position	Percentage of time spent for pigs facing the trough in relation to to the total time of the test.
Time spent in back position	Percentage of time spent for pigs facing the door opposite the trough in relation to the total time of the test.
Time spent in side position	Percentage of time spent for pigs crossed perpendicular to the door and the trough in relation to the total time of the test.
Locomotive Behaviour	
General Activity	Number of white lines marked on the floor crossed by both forelimbs of pigs.
Reluctant to Move	When the pig stopped for at least 2 s without showing exploratory behaviour.
Turning Back	Quick change of the body in opposite direction to the trough.
Retreat Attempts	When pigs backed away from the trough.

417 [1]Based on Erhard et al., (1999), Beattie et al., (1995), Boissy (1995) and Von Borell and
418 Ladewig (1992)
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427 **Table 3.** Comparison between control and treatment sessions of feeding (in percentage
428 of animals, mean ± SE), approaching (in percentage of time spent, mean ± SE) and
429 locomotive behaviour (in number of white lines crossed or events registered, mean ±
430 SE) by groups (midazolam and saline) in post-weaning and finishing pigs.

		POST-WEANING PIGS			FINISHING PIGS		
		Control	Treatment	Significance[1]	Control	Treatment	Significance[1]
Feeding	Saline	48.6 ± 2.35	37.1 ± 10.17	NS	68.3 ± 4.36	42.2 ± 8.46	*
behaviour (%)	Midazolam	59.1 ± 4.36	85.7 ± 5.71	*	43.5 ± 3.10	64.4 ± 9.29	*
Approaching behaviour							
Time on the	Saline	30.4 ± 5.24	17.8 ± 4.25	*	33.1 ± 4.46	16.3 ± 2.43	*
trough area (%)	Midazolam	31.0 ± 4.87	72.3 ± 5.29	*	23.0 ± 3.83	45.6 ± 6.06	*
Time on the	Saline	27.9 ± 3.78	34.7 ± 4.20	NS	18.0 ± 2.42	31.9 ± 4.76	*
distant area (%)	Midazolam	25.0 ± 3.47	10.2 ± 3.05	*	26.3 ± 3.14	20.1 ± 3.78	*
Time in a head	Saline	50.5 ± 4.51	57.5 ± 3.76	NS	59.3 ± 3.71	62.8 ± 3.58	NS
position (%)	Midazolam	54.4 ± 3.71	84.4 ± 3.68	*	52.5 ± 3.61	77.0 ± 3.62	*
Time in a back	Saline	40.8 ± 4.01	32.5 ± 3.38	*	36.7 ± 3.50	25.3 ± 3.12	*
position (%)	Midazolam	33.1 ± 3.05	12.4 ± 2.95	*	40.5 ± 3.53	16.0 ± 2.67	*
Time in a side	Saline	8.6 ± 1.27	9.9 ± 1.39	NS	4.1 ± 0.76	11.9 ± 1.75	*
position (%)	Midazolam	12.6 ± 1.70	3.2 ± 0.96	*	5.2 ± 1.75	6.9 ± 1.49	NS
Locomotive behaviour							
General Activity	Saline	27.4 ± 2.60	31.6 ± 2.44	NS	24.0 ± 2.81	26.5 ± 2.73	NS
	Midazolam	33.5 ± 3.77	12.0 ± 2.61	*	22.1 ± 2.09	13.4 ± 2.72	*
Reluctant to	Saline	3.3 ± 0.73	2.4 ± 0.26	NS	1.8 ± 0.28	2.0 ± 0.29	NS
Move	Midazolam	3.1 ± 0.62	0.6 ± 0.18	*	1.7 ± 0.32	0.4 ± 0.13	*
Turning Back	Saline	1.4 ± 0.38	1.6 ± 0.22	NS	1.2 ± 0.24	2.7 ± 0.25	*
	Midazolam	2.1 ± 0.39	0.42 ± 0.15	*	1.1 ± 0.25	1.1 ± 0.20	NS
Retreat	Saline	0.24 ± 0.13	1.4 ± 0.22	*	0.7 ± 0.12	1.7 ± 0.27	*
Attempts	Midazolam	0.3 ± 0.13	0.4 ± 0.14	NS	0.5 ± 0.27	0.8 ± 0.27	NS

431 [1] NS: no significant differences; *: significant differences at $P < 0.05$.

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437 **Table 4.** Comparison between saline and midazolam pigs of feeding (in percentage of
438 animals, mean ± SE), approaching (in percentage of time consumed, mean ± SE) and
439 locomotive behaviour (in number of white lines crossed or events registered, mean ±
440 SE) by stimuli (auditory, olfactory and visual) and comparison between stimuli for each
441 group of pigs (saline and midazolam) in post-weaning and finishing pigs.
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		POST-WEANING PIGS			FINISHING PIGS		
		Saline	Midazolam	Significance[1]	Saline	Midazolam	Significance[1]
Feeding behaviour (%)	Auditory	50.0 ± 10.00	100.0 ± 0.00	*	33.3 ± 13.33	80.0 ± 11.55	*
	Olfactory	10.0 ± 10.00	70.0 ± 10.00	*	46.7 ± 11.33	53.3 ± 13.33	NS
	Visual	30.0 ± 10.00	80.0 ± 0.00	*	40.0 ± 20.00	60.0 ± 23.09	NS
Approaching behaviour							
Time on the trough area (%)	Auditory	31.1 ± 12.00^{a}	78.5 ± 15.41	*	11.7 ± 9.16	67.9 ± 14.59	*
	Olfactory	9.3 ± 7.42^{b}	69.7 ± 11.97	*	23.9 ± 10.15	54.7 ± 17.01	*
	Visual	19.9 ± 10.44^{ab}	94.5 ± 11.57	*	23.1 ± 10.79	41.6 ± 14.47	NS
Time on the distant area (%)	Auditory	31.2 ± 4.17	17.5 ± 9.04^{ab}	*	57.3 ± 9.21^{a}	20.9 ± 9.73	*
	Olfactory	47.2 ± 8.46	23.7 ±6.35^{a}	*	30.9 ± 8.91^{b}	29.9 ± 10.70	NS
	Visual	36.0 ± 11.14	6.7 ± 5.93^{b}	*	26.7 ± 8.98^{b}	21.6 ± 8.75	NS
Time in a head position (%)	Auditory	57.2 ± 5.58	90.3 ± 4.47	*	52.6 ± 5.67^{b}	77.2 ± 6.69	*
	Olfactory	58.2 ± 6.96	91.7 ± 4.20	*	62.7 ± 6.80^{ab}	72.7 ± 6.96	NS
	Visual	57.1 ± 7.38	71.2 ± 8.45	NS	72.7 ± 6.39^{a}	81.3 ± 5.19	NS
Time in a back position (%)	Auditory	10.9 ± 2.28	0.8 ± 0.39^{b}	*	12.7 ± 3.13	6.8 ± 2.68	NS
	Olfactory	7.1 ± 1.61	1.9 ± 1.00^{b}	*	9.4 ± 2.00	7.1 ± 3.00	NS
	Visual	11.9 ± 3.10	7.0 ± 2.44^{a}	NS	13.9 ± 3.76	7.0 ± 2.18	NS
Time in a side position (%)	Auditory	31.9 ± 5.58	8.9 ± 4.21^{b}	*	34.7 ± 4.99^{a}	16.0 ± 4.51	*
	Olfactory	34.7 ± 6.40	6.3 ± 3.43^{b}	*	27.8 ± 5. 71^{ab}	20.2 ± 5.50	NS
	Visual	31.0 ± 5.93	21.8 ± 6.54^{a}	NS	13.3 ± 4.21^{b}	11.7 ± 3.78	NS
Locomotive behaviour							
General Activity	Auditory	23.0 ± 6.24^{b}	10.4 ± 5.34	NS	28.2 ± 3.50	13.3 ± 3.39	*
	Olfactory	44.7 ± 4.40^{a}	17.3 ± 4.43	*	27.8 ± 4.16	13.7 ± 4.09	NS
	Visual	26.9 ± 3.84^{b}	8.3 ± 3.82	*	23.6 ± 4.56	13.1 ± 4.16	NS
Reluctant to Move	Auditory	2.8 ± 0.41	0.6 ± 0.37	*	2.4 ± 0.39	0.3 ± 0.31	*
	Olfactory	1.7 ± 0.33	0.9 ± 0.37	*	1.6 ± 0.32	0.5 ± 0.31	*
	Visual	2.8 ± 0.39	0.2 ± 0.31	*	2.1 ± 0.46	0.6 ± 0.32	*
Turning Back	Auditory	1.1 ± 0.23^{b}	0.4 ± 0.27^{b}	*	1.2 ± 0.14^{a}	0.3 ± 0.22	*
	Olfactory	2.7 ± 0.35^{a}	0.7 ± 0.37^{a}	*	1.1 ± 0.25^{a}	0.1 ± 0.18	*
	Visual	1.1 ± 0.23^{b}	0.2 ± 0.17^{b}	*	0.6 ± 0.39^{b}	0.1 ± 0.26	NS
Retreat Attempts	Auditory	1.1 ± 0.29^{b}	0.2 ± 0.27^{b}	*	1.5 ± 0.36^{b}	0.3 ± 0.23^{b}	*
	Olfactory	1.4 ± 0.31^{ab}	0.5 ± 0.33^{ab}	*	1.6 ± 0.42^{b}	1.6 ± 0.34^{a}	NS
	Visual	1.8 ± 0.32^{a}	0.6 ± 0.26^{a}	*	2.0 ± 0.58^{a}	1.5 ± 0.47^{a}	NS

462 [1] NS: no significant differences; *: significant differences at $P < 0.05$. Different letters
463 (a-b) means significant differences for the comparison between stimuli at $P< 0.05$.
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466 **Figure. 1**. Test facilities and housing pens.

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