

Effect of supplementation with MgCO₃ and L-tryptophan on the welfare and on the carcass and meat quality of two halothane pig genotypes (NN and nn)

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Abstract

Sixty-one animals with different Halothane genes (homozygous halothane positive, n=34; and homozygous halothane negative, n=27) were fed with three diets (control group, with no supplement; magnesium (Mg) group with 1.28g MgCO₃/kg and tryptophan (Trp) group with 5g L-Trp/kg) during the last 5 days before slaughter. Animals were submitted to minimal stress *ante mortem* conditions. Pig behaviour was recorded at the experimental farm, raceway to the CO₂ stunning system and during the stunning period. Corneal reflexes were recorded after stunning as well. There were no differences in feed intake among diets (p>0.05) during the 5 days of treatment. The halothane positive (nn) group had lower intake than the halothane negative (NN) group (p<0.01). The behaviour of the pigs in the raceway did not differ (p>0.05) among treatments or halothane genotype. A significant (p<0.001) interaction diet*halothane was found in the time to appear the first retreat attempt during the exposure to the CO₂ system. In the nn group, the time of performing the first retreat attempt was later in the Mg (p<0.05) than the Control group. Moreover, in the Mg group, the nn had a later (p<0.05) first retreat attempt than the NN. Thus, Mg supplementation could have a positive effect on welfare of nn pigs. The nn had a lower proportion of animals that showed corneal reflexes after stunning than NN, indicating a higher effectiveness of the stunning method in nn pigs. Neither Mg nor Trp affected carcass quality and meat quality parameters, although significant differences were found between genotypes.

1.1 Introduction

The pre-slaughter handling of fattening pigs is one of the most stressful events and it has an important effect on the final carcass and pork quality (Gispert *et al.*, 2000a). It includes mixing unfamiliar animals, fasting, loading, transport, abattoir lairage and the stunning procedure. All of these are stressful factors which sometimes are difficult to alleviate. The most unpleasant situation for the pigs takes place during handling and transport before slaughter (Gispert *et al.*, 1996). Inappropriate handling results in lower animal welfare shown by skin damage and a higher mortality among the pigs. Moreover, it can lead to poorer pork quality such as PSE (*Pale Soft and Exudative*) and DFD (*Dark Firm and Dry*) meat.

Likewise, the stunning procedure used should be considered because it may modify the final carcass and pork quality (EFSA, 2004). In some countries, including Spain, the CO₂ stunning method has increased in popularity due to its positive effects on meat quality compared with the electrical method (Velarde *et al.*, 2001). In the CO₂ stunning dip-lift system, pigs are lowered directly into the maximum carbon dioxide concentration at the bottom of the pit inducing their unconsciousness (EFSA, 2004). It is a statutory requirement in Europe to use a minimum of 70% CO₂ (Directive 93/119/EC), however many slaughterhouses use between 83 and 90% CO₂ to increase throughput. Nevertheless, the use of carbon dioxide is questioned on welfare grounds because it has been described as an aversive gas (Raj & Gregory, 1995), causing irritation to the nasal mucosal membranes and lungs (Peppel & Anton, 1993), a sense of breathlessness and in many pigs it provokes escape attempts (EFSA, 2004).

The loss of consciousness is not immediate and several indicators of aversion during the induction of unconsciousness with CO₂ has been described in pigs (Raj & Gregory, 1995), such as escape attempts or retreat attempts (Dodman, 1977). Therefore, the stunning procedure can be considered as a stressful pre-slaughter stimulus and should be considered from the animal welfare and pork quality point of view.

Acute pre-slaughter stress may result in an increase in drip loss and paler meat (Grandin, 1980), producing PSE meat. The existence of PSE meat also has a genetic origin related to the halothane gene (n), also known as RYR1 gene. In fact, homozygous halothane positive (nn) and heterozygous (Nn) pigs have an abnormality in their muscle metabolism that makes them particularly sensitive to acutely stressful stimuli, causing a higher incidence of PSE meat (Jensen & Barton-Gade, 1985 and Oliver *et al.*, 1993).

This abnormality does not exist in halothane negative (NN) pigs. Gispert *et al.* (2000b) stated that the frequency of the halothane gene (n gene, considering NN, Nn and nn pigs) in Spanish pigs was 32%, so that the removal of the Halothane gene (Nn and nn) from the Spanish pig population would decrease the incidence of PSE meat by 65% (Gispert *et al.*, 2000a). Furthermore, an eleven-fold reduction in mortality rates could be expected (Fàbrega *et al.*, 2002). However, at the moment halothane carriers are present amongst the Spanish slaughter pig population, because carcass conformation is considered a relevant trait for ham production. Accordingly, it is necessary to find a strategy to reduce the effects of acute pre-slaughter stress.

Stress associated effects on animal welfare and on pork quality may be reduced by including feed components such as Mg or Trp in the diet. Mg is reported to be an important cofactor in many enzymatic reactions involved in energy and protein metabolism and can counteract catecholamine effects in stress situations, and thus decrease the acute stress response resulting from handling prior to slaughter (Kietzmann & Jablonsky, 1985). The primary effect of Mg appears to be a reduction in neuromuscular stimulation due to its calcium antagonist effects (Rosenvold & Andersen, 2003), so it may control intracellular calcium (Laver *et al.*, 1997). Peeters *et al.* (2005) reported that pigs subjected to vibration in a transport simulator after a Mg supplementation spent more time lying down. It may also delay the initiation of glycolysis by maintaining high energy phosphates *post-mortem* (Moesgaard *et al.*, 1993). Indeed, short-term supplemental Mg has been reported to improve pork water holding capacity (WHC), pork colour and pork texture (D'Souza *et al.*, 1998; Caine *et al.*, 2000; Frederick *et al.*, 2006). However, different results have been obtained when comparing the effect of Mg supplementation to carriers (Nn) and non-carriers (NN) of the Halothane gene (Apple *et al.*, 2000; Caine *et al.*, 2000).

The inclusion of Trp in the diet increases plasma Trp, brain Trp and brain serotonin concentration (Fernstrom & Wurtman, 1971) so it may affect mood regulation, feed intake, behaviour, and sleep patterns (Leathwood, 1987). Trp supplementation may also improve muscular pH (Henry *et al.*, 1996) and reduce the incidence of PSE meat (Adeola & Ball, 1992 and Henry *et al.*, 1996). However, the evidence of a significant impact of Trp on meat quality is not conclusive. Guzik *et al.* (2006) reported poorer meat quality after a Trp supplementation although Li *et al.* (2006) found no significant effect when the Trp supplementation was used.

The aim of this study was to assess the effect of dietary supplementation with Mg and Trp on animal behaviour and welfare and on carcass and meat quality of pigs (NN and nn), in an experimental study carried out under non-stressful *ante-mortem* conditions. The study was carried out with NN and nn pigs because as mentioned previously a considerable percentage of Nn pigs are still present in the slaughter pig population in Spain. Therefore the study aimed at evaluate whether the use of natural tranquilizers could have a different effect depending on the genotype of the pigs.

1.2 Materials and methods

1.2.1 *Animals and diets*

Sixty-one entire male pigs were selected from a group of 72, after a DNA test (34 homozygous negative–NN- and 27 homozygous positive–nn- with respect to Halothane gene) from Landrace, Large White and Pietrain lines (average live weight: 102.6 ± 12.47 kg). They were housed in 6 pens according to Halothane genotype (NN or nn) and dietary supplements (3 diets) in the experimental farm (IRTA-Monells, Spain). They were fed the same commercial diet (30% barley, 25% soy, 15% maize, 12% wheat and a vitamin/mineral source) until 5 days before slaughter. The diet contained 87.9% of dry matter, 18% of crude protein and 14.1 MJ/kg of digestible energy on fresh matter.

Five days before slaughter 3 diet groups were established for each genotype: control group, with no supplement; Mg group, same diet supplemented with 1.28g MgCO₃/kg (0.38% Mg); and Trp group, same diet supplemented with 5g L-tryptophan per kg (0.61% Trp). All pigs were allowed ad libitum access to feed and water through one single-hole self-feeder.

1.2.2 *Ante-mortem treatment and slaughter procedure*

The animals were submitted to the following *ante mortem* conditions: they were transported without mixing groups in the lorry according to diet and genotype, taking 15 minutes from the farm to the experimental abattoir. The lairage time was from 1 to 3 hours. They were stunned with 90% CO₂ and slaughtered. Exsanguination, scalding, dehairing, and evisceration were performed according to standard procedures used in IRTA-Monells.

1.2.3 *Animal welfare and behaviour measurements*

During the stunning procedure. The animals from the 6 groups were slaughtered alternatively. Pigs were randomly separated, placed individually in the corridor and allowed to cross the corridor voluntarily. If after one minute the animal was reluctant to move, it was gently pushed into the crate.

The raceway was 412 cm long and 60 cm wide, allowing ease of movement but stopping the animal from turning around. It was also lined with steel panels of 90 cm height to prevent the animal from seeing out of the raceway. The gate of the CO₂ stunning system consists of a non-slip steel ramp of 148 cm length and a slope of 7° with a guillotine gate entrance at the end. The behaviour of each animal in the raceway was recorded with a video camera (CCD-TR820; Sony) located at the raceway entrance. The CO₂ stunning unit consisted of a dip-lift system (Butina Aps, Copenhagen, Denmark) that contained a crate 195 cm long, 90 cm high and 61 cm wide, with perforated floor to facilitate the distribution of the gas inside. After the pig entered the crate, the gate closed behind it and descended to the bottom of the pit taking 23 s, remained stationary for 86s and then ascended taking 23 s. The total cycle lasted 132 s. The behaviour of the pigs during the descent into the pit was also recorded with a second video camera (CCD-TR820; Sony) placed on the roof of the crate.

Behavioural parameters were scored in the raceway: presence of retreat attempts (when the pig backed away; Dodman, 1977), voluntary movement (the handling of the animals was scored as 0 if the pig moved voluntarily or 1 if the animal was gradually forced to enter the crate) and time taken to cross the raceway and enter to the crate.

During the exposure to the CO₂, behavioural parameters were also scored to determine the aversion to the gas: presence of retreat attempts, first retreat attempt (time to perform the first retreat attempt in the CO₂ unit), presence of gasp (number of very deep breaths through a wide open mouth, which may involve stretching of the neck; which was considered to be an indicator of the onset of breathlessness; Lambooij *et al.*, 1999), first gasp (time to perform the first gasp), presence of escape attempts (number of fast running movements across the stunning box and sometimes raising their forelegs on the side of the wall of the crate either prior to, or at the time it was losing its posture; Raj & Gregory, 1996), first escape attempt (time to perform the first escape attempt) and time of loss of posture (it was considered when the animal fell in the crate, and was recorded as the first indicator of onset of unconsciousness; Raj & Gregory, 1996).

All recording times were synchronised with the time the pigs started to descend into the well.

After the stunning procedure, corneal reflexes were recorded by touching the cornea of the open eye with a pencil at 15-second intervals until one minute after ending the stunning procedure. If the eyelid closed when the eye was touched with a pencil, the corneal reflex was recorded as a positive, indicating that the brainstem was responsive (EFSA, 2004). The stun-stick interval was 23.3 ± 2.43 s.

1.2.4 *Chemical analysis of feed*

Feed was analyzed for Mg and Trp concentration in all diets (Table IV.2-1).

1.2.5 *Measurements of carcass and meat quality*

The carcass weight (kg) was determined individually. Carcass grading was carried out using the Fat-o-Meater grading probe (FOM) in the left side of the carcass. Carcass lean percentage was estimated using the Spanish official equation (Gispert & Diestre, 1994).

On line measurements. The left side of the carcass was used to perform meat quality measurements on the *Longissimus thoracis* (LT) and *Semimembranosus* (SM) muscles. Muscle pH at 45 min (pH45) and at 24h *post-mortem* (pHu) was measured using a portable pH-meter KNICK equipped with a Xerolyt electrode. Electrical conductivity at 24h *post mortem* (ECu) was measured at the last rib level using a Pork Quality Meater (PQM-I, INTEK Aichach, Germany). Muscles (LT and SM) showing $\text{pH}_{45} \leq 5.8$ were classified as PSE meat (Honkavaara, 1988), whereas those presenting pHu values ≥ 6.00 were classified as DFD (Oliver *et al.*, 2001).

At 24h *post mortem* the loin was removed from the left side of each carcass to take samples, which were vacuum-packed in aluminium bags and frozen at -20°C until analysis.

Drip loss measurements. Muscle samples from the LT muscle were collected at 24h *post-mortem*, at the 3/4 last rib level in the cranial direction, to determine drip losses, following the reference method supported by OECD (Honikel, 1996).

Meat colour. Colour measurements were carried out at the last rib section with a Spectrophotometer Minolta 2002 on the CIELab space (CIE, 1976) using illuminant D65 and 10° standard observer.

Intramuscular fat. Intramuscular fat was analysed by Near Infrared Transmittance (NIT, Infrared, 1265, Tecator) spectroscopy (Gispert *et al.*, 1997) in the LT muscle..

Cathepsin activity. Samples for Cathepsin activity measurements were taken in the LT and SM muscle at 24h *post-mortem*. After removing the subcutaneous fat and connective tissue, muscles were ground and kept at -20 °C until further analysis. Cathepsins were extracted according to the method of Etherington *et al.* (1990). Cystein proteinases B and L were assayed fluorimetrically using the method of Etherington & Wardale (1982). One unit activity was defined as the amount of enzyme hydrolysing 1 nmol of substrate min⁻¹ at 37 °C. Protein concentration of the enzymatic extracts was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

Texture analysis. Instrumental tenderness was determined on boneless loin chops (*Longissimus thoracis*). Pork chops were thawed for 24h at 4 °C in their vacuum-packed aluminium bag and then cooked in a convection oven pre-heated to 110 °C to an internal temperature 75 °C. Chops were allowed to come to room temperature before a minimum of six pieces 3.0x1.5x1.5 cm were removed per chop. All pieces were sheared using a MTS Alliance RT/5 texture analyzer (MTS System Corp., Eden Prairie, MN, USA) equipped with a Warner Bratzler blade with crosshead speed set at 2 mm/s, and peak load (kg), modulus (kg.mm⁻¹) and peak energy (kg.mm) were recorded.

1.2.6 *Statistical analysis*

Statistical analysis was performed using the computer software Statistical Analysis System (SAS, 2001).

Animal welfare observations are count data (number of retreat attempts), time intervals (time taken to enter the crate, time to first retreat, time to first gasp, time to loss of posture, time to onset of escape attempts) and binary data (voluntary movement, presence of retreat attempts and presence of escape attempts). A Chi Square test was applied to compare the distribution of the frequencies of behavioural parameters (count data) according to the halothane genotype or the diet. In the case of the number of observations being less than 5, the Chi Square could not be performed. In these cases, count data were analysed by the PROC GLM procedure. The fixed effects included in the model were genotype, diet and their interaction. Differences among diets and genotypes were tested using the Tukey test.

Carcass parameters and meat quality parameters were analysed using the GLM procedure. Genotype and diet were considered as fixed effects (the interaction was not significant in any of the carcass and meat quality variables studied). The day effect was

included in the model as a blocking effect and carcass weight was included as a covariate when it was significant. Differences among diets or genotypes were tested using the Tukey test. Significance was fixed at $P < 0.05$.

The experiment was approved by the Institutional Animal Care and Use Committee (IACUC) of IRTA.

1.3 Results

1.3.1 *Animal behaviour and welfare in the experimental abattoir*

Behaviour of the pigs in the raceway is presented in Table IV.2-2. No significant differences ($P > 0.05$) were found among diets or genotypes in percentage of pigs showing retreat attempts, moving voluntarily to the crate or in the time to cross the raceway.

As shown in Table IV.2-3, no significant differences ($P > 0.05$) were observed among diets or genotypes in behavioural parameters evaluated in pigs in the CO₂ stunning unit. The incidence of animals showing retreat attempts, gasping and escape attempts did not differ among treatments ($P > 0.05$). Furthermore, no differences were observed among treatments or genotypes in the time to perform the first gasp, the first escape attempt and the duration of them. The behaviour observed during the CO₂ exposure is shown in figure IV.2-1. The first behavioural parameter observed was the time to perform the first retreat attempt (4.55 ± 3.167 s). Pigs showed the first gasp at 11.28 ± 3.728 s and they lost their posture at 22.85 ± 4.638 s.

A significant interaction ($P < 0.001$) among diets and genotypes was found in the time to perform the first retreat attempt (Table IV.2-4). In nn animals, the time to perform the first retreat attempt was longer in Mg group than in Control one ($P < 0.05$), whereas in NN pigs the opposite result was obtained: pigs from Mg group took less time to perform the first retreat attempt than those from Control group ($P < 0.05$). In both cases Trp diet was in-between.

Comparing diets within genotypes, in control diet nn pigs went back earlier than NN ones ($P < 0.05$). The opposite behaviour was observed in Mg diet: nn animals took more time to perform the first retreat attempt ($P < 0.05$) than NN. Finally, no differences ($P > 0.05$) were found between NN and nn genotypes when a Trp diet was used.

At the end of the CO₂ exposure, presence of corneal reflexes was monitored for one minute to determine whether the pig was still losing consciousness, was already in an

unconscious state or was regaining consciousness (Figure IV.2-1). No significant differences ($P>0.05$) among diets were observed in the incidence of animals showing corneal reflexes, in any of the observations carried out at 0, 15, 30, 45 and 60s after the stunning procedure. With respect to HAL gene, a higher percentage ($P<0.05$) of NN animals showed corneal reflexes in comparison with nn ones (32.4% vs. 7.4%). Furthermore, the NN group had a higher presence of corneal reflexes at 0s (Figure IV.2-2) but no differences were observed between 15 and 60s ($P>0.05$).

1.3.2 *Carcass and meat quality*

There was no significant diet x genotype interaction ($P>0.05$) in any carcass and pork quality measurements on the LT or SM. Accordingly, only the main effects are reported.

No significant effects were found among diets on carcass and meat quality parameters ($P>0.05$). However, differences ($P<0.001$) were found between genotypes in carcass and meat quality traits. The NN genotype had longer carcasses (83.87 vs. 76.35%), higher conformation (3.3 vs. 1.1) and lower lean content (55.3 vs. 60.5%) than the nn genotype. Furthermore, in comparison to NN pigs, nn pigs had lower pH₄₅ in both muscles (5.59 vs. 6.46 in LT muscle; 5.78 vs. 6.40 in SM muscle), higher electrical conductivity (7.8 vs. 4.4 in LT muscle; 8.8 vs. 4.3 in SM muscle) and higher loin drip loss (10.47 vs. 5.78) and, therefore, these animals showed a higher incidence of PSE than NN pigs (74.1 vs. 8.8% in LT muscle and 96.3 vs. 11.8% in SM muscle).

Enzymatic activity was not affected by diet, but a significant genotype effect was observed. In general, nn pigs had higher enzymatic activity than NN pigs (Table IV.2-5). Similar results were found when instrumental texture variables are considered. No differences among diets ($P>0.05$), but significant differences between genotypes were found ($P<0.05$; Table IV.2-5), being the meat of the nn pigs harder than the meat from NN ones.

1.4 Discussion

1.4.1 *Animal behaviour in the experimental abattoir. Aversion to the exposure to the CO₂*

In the experimental abattoir, no significant differences were found among diets and between genotypes in the time taken to cross the raceway and enter the stunning system.

Furthermore, in general, no differences were found during the stunning procedure even though a diet-by-genotype interaction was observed in the time taken to perform the first retreat attempt in the crate. It is known that when pigs are confronted with an unpleasant situation, such as the CO₂ stunning procedure, the first reaction is to back away (Dodman, 1977). Although no differences among diets and genotypes were found, the presence of retreat and escape attempts indicated aversion to the exposure of a high concentration of CO₂ (Raj & Gregory, 1995; Velarde *et al.*, 2007). Furthermore, although gasping is not considered an expression of aversion, it may compromise animal welfare as it is a physiological reaction associated with breathlessness during the inhalation of the gas (Raj & Gregory 1996; Lambooij *et al.* 1999).

Velarde *et al.* (2007) stated that pigs carrying the halothane gene (Nn) were relatively more sensitive to exposure to CO₂, showing a higher incidence of aversive behaviour than halothane negative pigs (NN). Although the present study was performed using halothane positive (nn) and free-gene halothane negative pigs (NN), no differences were observed between them. The halothane gene is linked to a higher susceptibility to stress (Fàbrega *et al.*, 2002), but under the minimal stress conditions used in the present study, differences between NN and nn pigs could be difficult to identify.

Halothane carriers are still present amongst the Spanish slaughter population because carcass conformation is considered a relevant trait for ham production. According to our results, Mg supplementation may have a positive effect on nn pigs, because it delays the time of performing the first retreat attempt with respect to the nn control group. However, Mg could have a negative effect on NN pigs because NN pigs from the Mg group performed the first retreat attempt earlier than the NN control ones. Therefore, the efficacy of an Mg supplementation may be dependent on pigs' genotype. To our knowledge, there is no information available on the effect of Mg or Trp during the CO₂ stunning procedure.

It is well established that nn pigs have a mutation in the RYR1 gene which codifies the calcium release channel of the sarcoplasmic reticulum by the skeletal muscle (Fuji *et al.*, 1991). The mutation is related to an increase in the intracellular calcium levels, and Mg (from the Mg supplementation) may control the release of this intracellular calcium (Laver *et al.*, 1997).

The stunning procedure before slaughter is performed to induce unconsciousness and insensibility in animals so that slaughter can be performed avoiding, as much as possible, animal suffering. The loss of consciousness is gradual until it reaches

anaesthesia deep enough to be slaughtered without experience pain or stress. In the present study the absence of corneal reflexes was used to assess the effectiveness of the CO₂ stunning in Mg and Trp groups. The corneal reflex is generally the last reflex to disappear during the loss of consciousness and it is likely that the animal is not sensible to pain when they are absent (EFSA, 2004). At the exit of the stunning, still 20% of the pigs showed corneal reflexes. This reflex disappeared up until 40 seconds from the exit of the stunning procedure. So the anaesthesia of the pigs became deeper after the stunning and they all reached maximum unconsciousness after 40 seconds, which could be considered the best moment to kill them. Although no differences among diets were observed regarding the presence of corneal reflexes, differences between genotypes were found. Our results indicated that the consciousness was lighter for the NN pigs than nn ones, so the probability of recovery from unconsciousness before death was higher in NN than in nn. In agreement with our results, Velarde *et al.* (2007) reported a reduction of the onset of unconsciousness in pigs containing the halothane gene (Nn) in comparison to NN.

Exposure to carbon dioxide induces hyperventilation in pigs before the onset of unconsciousness (Raj & Gregory, 1996). Forslid (1992) reported that this faster and deeper respiration is an advantage because it facilitates uptake of CO₂ and shortens the induction of unconsciousness, which could be an advantage from an animal welfare point of view. On the other hand, Schaefer *et al.* (1987) suggested that halothane positive pigs displayed a higher hypercapnic condition characterized by high venous levels of CO₂, bicarbonate and base excess than either Nn or NN genotypes. Therefore, either the increase in respiratory frequency or the increase of blood CO₂ may induce unconsciousness earlier in nn pigs than in NN pigs.

1.4.2 *Carcass and meat quality*

Some discrepancies exist in previous works with respect to the effect of dietary Mg supplementation when comparing carriers and non-carriers of the halothane gene (Caine *et al.*, 2000). Schaefer *et al.* (1993) pointed out that stress-susceptible pigs (in particular, heterozygous carriers of the halothane gene) may benefit the most from the supplementary Mg. Since the interaction between genotypes and diets was not significant, no differences between NN and nn pigs were found in the effect of Mg and Trp on carcass quality.

The length of the supplementation seems to be critical for the carcass and meat quality. Supplemental Mg or Trp in the diets of growing-finishing pigs during 5 days had no appreciable effect on carcass traits. A five-day treatment with Mg or Trp was not enough to improve carcass traits such as yield and lean content. This is consistent with the observations reported by Hamilton *et al.* (2002) and Adeola & Ball (1992) when a short term supplementation with Mg or Trp was used. In contrast, Apple *et al.* (2000) reported an increase of lean content after a long-term supplementation when magnesium mica was included in the starter, grower and finisher diets. Trp has been shown to increase protein synthesis in the liver of pigs (Cortamira *et al.*, 1991) but feeding crystalline Trp to pigs above their perceived nutritional requirement has not been shown to have a beneficial effect on growth performance (Adeola & Ball, 1992). Therefore, given the short time period of supplementation (one week) it is unlikely that Mg or Trp significantly increases protein or lipid deposition and thus probably does not affect carcass yield. On the other hand, significant differences were found between genotypes as was expected. The halothane gene has been associated with a higher carcass lean content and better conformation in nn pigs compared to NN ones, due to lower fat and bone proportions and better carcass weight distribution (Oliver *et al.*, 1993).

In agreement with our results, some authors did not find any improvement in pH₄₅ using Mg short-term supplementation (Frederick *et al.*, 2006) even though others suggested an improvement of initial pH values and of pork colour, and a reduction of the drip losses and of the incidence of PSE pork (Schaefer *et al.*, 1993; D'Souza *et al.*, 1998; Hamilton *et al.*, 2002).

On the other hand, regarding the use of dietary triptophan, results of the present study agree with those of Guzik *et al.* (2006) and Li *et al.* (2006) that indicated that the use this aminoacid is not beneficial to meat quality and it could even have a stimulatory effect instead of a sedative one. Adeola & Ball (1992) found a reduction of PSE pork in finishing pigs supplemented with Trp 5 days before slaughter. Therefore, further research is required to elucidate the effect of short-term Mg and _L-Trp supplementation. The negative effect of the halothane or RYR1 gene on meat quality has long been recognised. Accordingly, RYR1 gene significantly affected meat quality traits (initial muscle pH, electrical conductivity, colour and the incidence of PSE) with the exception of ultimate muscle pH (Jensen & Barton-Gade, 1985; Gispert *et al.*, 2000b).

There is no evidence that Mg supplementation may modify *post-mortem* proteolysis and tenderization (Schaefer *et al.* 1993; Panella *et al.*, 2005). Although Ertbjerg *et al.*,

(1999) found a relationship between the loin tenderness and the activity of proteolytic enzymes such as cathepsins, this relation was not found in the present study since correlations between cathepsins activity and shear force values were not significant (data not shown). In the present study, differences between genotypes were found in the cathepsin activities in the SM and LT muscle, in accordance with other authors that suggest a genetic component in the variability of the proteolytic enzyme (Hernández *et al.*, 2004; Plastow *et al.*, 2005). Similarly, shear force values may vary with the RYR1 gene, since nn pigs showed significantly higher values ($p < 0.05$) than NN pigs (Murray *et al.*, 1989; Moelich *et al.*, 2003). In contrast, De Smet *et al.* (1996) found no differences between these genotypes.

Although the study was performed under minimal stress conditions, the incidence of PSE meat was relatively high. This confirmed the negative effect of the RYR1 gene on meat quality traits, and suggested that the supplementation with natural tranquilizers should not be used as a substitute for good *ante-mortem* handling.

Further research is required to elucidate the effect of Mg and Trp on animal welfare, carcass and meat quality. Increased concentrations of Mg immediately before slaughter may improve meat quality by decreasing the acute stress response resulting from handling prior to slaughter (Kietzmann & Jablonsky, 1985). Accordingly, increasing Trp intake immediately before slaughter may also improve meat quality by increasing serotonin and reducing stress, and allowing the glycogen stores to remain at a high level. However, because of the fasting that commonly occurs before slaughter, the diet may not play an important role in affecting the stress response. Therefore, greater concentrations of dietary Mg or L -Trp, a combination of different tranquilizers or the supplementation via drinking water may be necessary to improve meat quality.

Further studies are required under commercial conditions or using different chemical compounds, or a combination of Mg and Trp to obtain a reduction in the incidence of PSE meat.

1.5 Conclusions

Supplementation of Mg in the diet of pigs could have a positive effect on animal welfare of nn pigs, decreasing the aversion to the CO₂ gas. On the other hand, the exposure to 90% of CO₂ during 132s induced unconsciousness in the nn pigs earlier than in the NN ones, indicating that the gas could be more effective on nn animals.

None of the treatments had an effect either on the carcass parameters (back fat thickness and lean content) or on the meat quality traits although they were affected by the halothane gene.

The supplementation of magnesium carbonate or Trp for 5 days before slaughter failed to show any beneficial effects on meat quality traits when minimal stress *ante-mortem* conditions were used.

1.6 References

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1.7 Figures

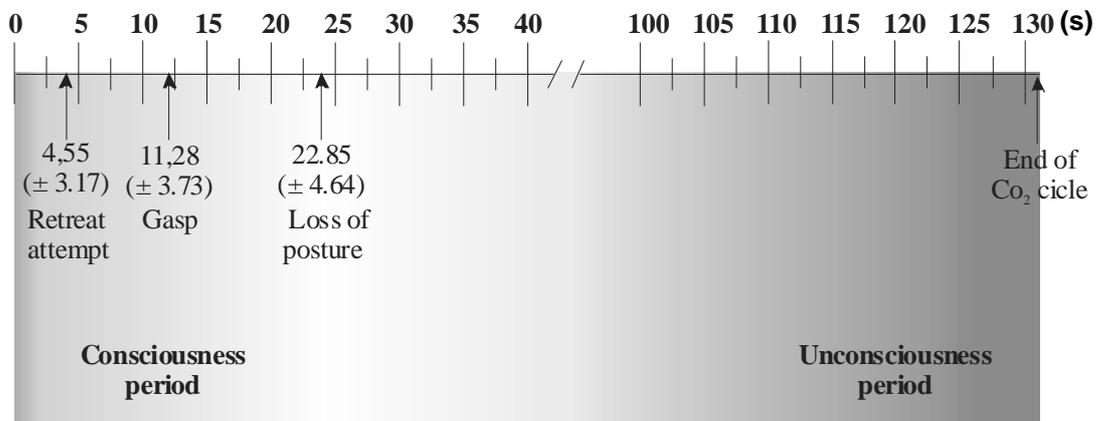


Figure IV.2-1. Pig behaviour during the exposure to CO₂ (90%, 132s). The values correspond to the least square means and standard error (in brackets) of the time (s) to perform the first retreat attempt, the first gasp and the loss of posture.

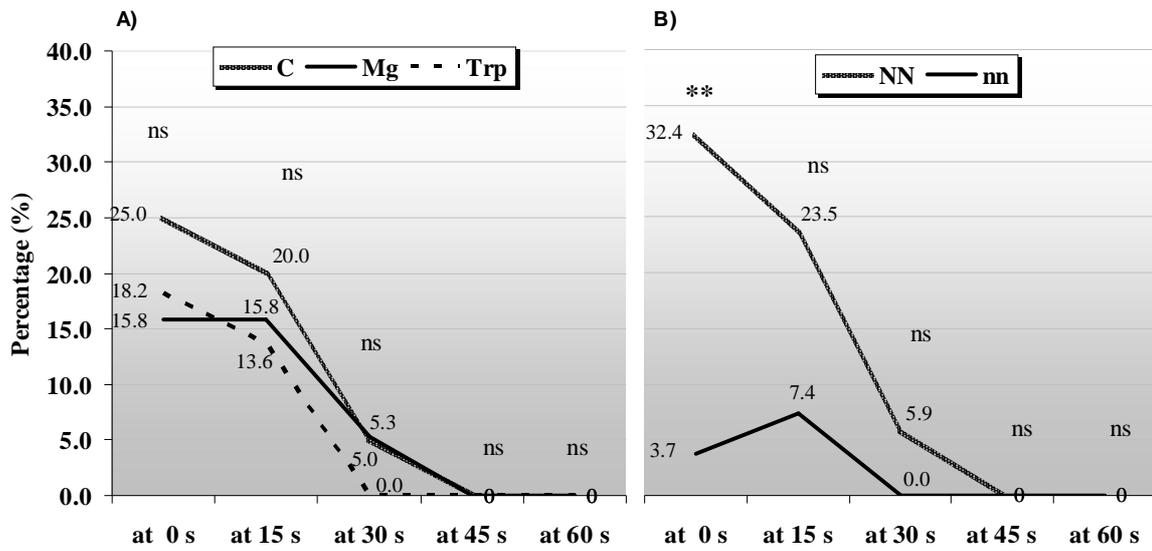


Figure IV.0-2. Incidence of pigs (%) showing corneal reflexes among diets (A) or halothane genotype (B). C: Control Diet; Mg: Control diet with Mg supplementation; Trp: Control diet with Trp supplementation; ns:P>0.05 and **P<0.01.

1.8 Tables

Table IV.0-1. Magnesium and tryptophan concentration (g/100g fresh diet) in the control and supplemented diets.

	Control	Mg Supplemented	Trp Supplemented
Magnesium (%)	0.21	0.38	0.21
Tryptophan (%)	0.20	0.20	0.61

Table IV.0-2. Percentage of pigs showing retreat attempts and voluntary movement and time taken to cross the raceway according to three diets and two genotypes.

	Diet				Genotype			
	C	Mg	Trp	Sig	NN	nn	Sig	
Retreat attempts (%)	45.0	57.9	50.0	ns	50.0	51.85	ns	
Voluntary movement (%)	52.6	50.0	45.4	ns	56.2	40.7	ns	
Time to cross the raceway † (s)	LSM	244.4	248.1	243.0	ns	234.9	255.46	ns
	SE	(36.87)	(31.76)	(28.72)		(23.82)	(25.93)	

C: Control Diet; Mg: Control diet with Mg supplementation; Trp: Control diet with Trp supplementation; ns: P>0.05.

† Least Square Means (LSM) and Standard Error (SE), in brackets.

Table IV.0-3. Aversion to CO₂: behavioural parameters evaluated on the pigs in the CO₂ stunning unit during the descent into the pit according to three diets and two genotypes.

		Diet			Sig	Genotype		
		C	Mg	Trp		NN	nn	Sig
Retreat attempts (%)		90.00	89.47	90.91	ns	91.18	88.89	ns
Gasp (%)		75.0	73.7	77.3	ns	79.4	70.4	ns
First Gasp [†] (s)	LSM	10.4	12.6	10.6	ns	11.88	10.57	ns
	SE	(0.94)	(1.00)	(0.88)		(0.70)	(0.84)	
Escape Attempts (%)		100.0	100.0	95.4	ns	97.1	100.0	ns
First Escape attempt [†] (s)	LSM	19.1	18.2	18.1	ns	18.1	18.9	ns
	SE	(0.70)	(0.72)	(0.68)		(0.54)	(0.60)	
Duration Escape attempts [†] (s)	LSM	3.9	3.9	5.1	ns	5.2	3.4	ns
	SE	(1.19)	(1.19)	(1.12)		(0.90)	(1.01)	
Loss of posture [†] (s)	LSM	23.1	22.0	23.2	ns	23.2	22.3	ns
	SE	(1.08)	(1.08)	(1.03)		(0.82)	(0.93)	

C: Control Diet; Mg: Control diet with Mg supplementation; Trp: Control diet with Trp supplementation; ns:P>0.05

[†] Least Square Means (LSM) and Standard Error (SE), in brackets.

Table IV.0-4. Least square means (LSM) and Standard error (S.E.) of the time to perform the first retreat attempt during exposure to 90% CO₂ in atmospheric air, among diets and halothane genotype.

	NN		nn		Sig ⁺
	LSM	S.E.	LSM	S.E.	
Control Diet	6.30 ^a	0.908	2.25 ^b	1.015	**
Magnesium Diet	3.10 ^b	0.908	6.86 ^a	1.086	*
Tryptophan Diet	4.00 ^{ab}	0.866	5.11 ^{ab}	0.957	<i>ns</i>

C: Control Diet; Mg: Control diet with Mg supplementation; Trp: Control diet with Trp supplementation; different letters within columns are significantly different ($P \leq 0.05$); *ns*, $P > 0.05$, * $P < 0.05$ and ** $P < 0.01$.

Table IV.0-5. Enzymatic activity variables † (cathepsin B and cathepsin B+L) and instrumental texture variables † after Warner-Bratzler Shear force test, among diets and halothane genotype.

	Diets				Genotype		
	C	Mg	Trp	Sig ⁺	NN	nn	Sig ⁺
<i>Enzymatic activity variables</i>							
CAT B (UE/ mg prot)							
SM	0.116 (0.0064)	0.11 (0.0066)	0.108 (0.0061)	ns	0.104 (0.0049)	0.12 (0.0055)	*
LT	0.050 (0.0032)	0.044 (0.0030)	0.046 (0.0028)	ns	0.041 (0.0023)	0.053 (0.0026)	***
CAT B+L (UE/ mg prot)							
SM	0.343 (0.0134)	0.332 (0.0139)	0.326 (0.0128)	ns	0.306 (0.0103)	0.362 (0.0116)	***
LT	0.261 (0.0168)	0.206 (0.0160)	0.227 (0.0148)	ns	0.214 (0.0123)	0.248 (0.0136)	ns
<i>Instrumental texture variables</i>							
Shear Force (N)	55.45 (3.062)	53.76 (3.181)	61.64 (2.920)	ns	51.98 (2.481)	61.91 (2.835)	*
Initial Yield Force (Ncm)	79.57 (4.004)	76.15 (4.117)	84.60 (3.817)	ns	74.74 (3.067)	85.48 (3.447)	*
Total Work (kg.s/cm ²)	7.38 (0.380)	7.01 (0.390)	8.08 (0.362)	ns	7.04 (0.291)	7.94 (0.327)	*

† Least Square Means and Standard Error (in brackets)

C: Control Diet; Mg: Control diet with Mg supplementation; Trp: Control diet with Trp supplementation; CAT B: cathepsin B activity; CAT B+L: cathepsin B+L activity; SM: *Semimembranosus* muscle; LT: *Longissimus thoracis* muscle; +: ns, P>0.05, *P<0.05 and ***P<0.001.