

Effect of magnesium sulphate and L-tryptophan and genotype on the feed intake, behaviour and meat quality of pigs

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Abstract

Sixty-nine entire male pigs with different halothane genotype (homozygous halothane positive – nn-, n=36; and homozygous halothane negative – NN-, n=33) were fed with a supplementation of magnesium sulphate (Mg) and/or L-tryptophan (Trp) in the diet for 5 days before slaughter. Animals were housed individually and were submitted to stressful *ante mortem* conditions (mixed in the lorry according to treatments and transported 1h on rough roads). Individual feed intake was recorded during the 5-day treatment. At the abattoir, pig behaviour was assessed in the raceway to the stunning system and during the stunning period by exposure to CO₂. Muscle pH, colour, water holding capacity, texture and cathepsin activities were determined to assess meat quality. The number of pigs with an individual feed intake lower than 2kg/day was significantly different among diets (P<0.05; Control: 8.7%; Mg&Trp: 43.5%; Trp: 17.4%) and they were considered to have inadequate supplement intake. During the *ante mortem* period, 15.2% of pigs included in the experiment died, and this percentage decreased to 8.7% in those pigs with a feed intake > 2kg/day, all of them from the stress-sensitive pigs (nn). In general, no differences were observed in the behaviour of pigs along the corridor leading to the stunning system and inside the CO₂ stunning system. During the stunning procedure, Trp diet showed shorter periods of muscular excitation than control and Mg&Trp diets. The combination of a stressful *ante mortem* treatment and Mg&Trp supplementation led to carcasses with high incidence of severe skin lesions. Different meat quality results were found when considering all pigs or considering only those with adequate supplement intake. In this later case, Trp increased pH₄₅ (6.15) vs Control diet (5.96) in the *Longissimus thoracis* (LT) muscle (P<0.05) and pH at 24h (Trp: 5.59 vs C: 5.47) led to a higher incidence of dark, firm and dry (DFD) traits in SM muscle (P<0.05). Genotype affected negatively all the meat quality

traits. Seventy-five percent of LT and 60.0% of the SM muscles from nn pigs were classified as pale, soft and exudative (PSE), while none of the NN pigs showed these traits ($P < 0.0001$). No significant differences were found between genotypes on the incidence of DFD meat.

Due to the negative effects observed in the Mg&Trp group in feed intake and carcass quality, the utilization of a mixture of magnesium sulphate and tryptophan is not recommended.

1.1 Introduction

Carcass and meat quality of finishing pigs can be affected by genetics as well as by the pre-slaughter handling. During this period, pigs experience some of the most stressful situations in their life (Gispert *et al.*, 1996; Guàrdia *et al.*, 2004). The effect of the stress on the muscle metabolism is especially dramatic on the pigs carrying the halothane gene, *n*: nn pigs have a mutation in the RYR1 gene which codifies one of the calcium release channels of sarcoplasmic reticulum: the ryanodine receptor (Fujii *et al.*, 1991). This mutation makes pigs particularly sensitive to acute stress, which is the main cause of death before slaughter (Barton-Gade *et al.*, 1998) and of the development of pale, soft and exudative meat (PSE; Jensen & Barton-Gade, 1985; Oliver *et al.*, 1993). Despite these negative effects, the halothane gene is still used in Spanish commercial herds (Oliver *et al.*, 1993) because it enables producers to target carcass specifications of high value according to the price payment grids based on carcass weight and lean content (Gispert & Font i Furnols, 2007). Nevertheless, meat from halothane-free pigs (NN) submitted to pre-slaughter stressful stimuli can also become PSE (Honkavaara, 1988).

The stunning procedure may also modify the final carcass and pork quality (Channon *et al.*, 2000; EFSA, 2004). The CO₂ stunning system is one of the approved methods of stunning pigs in Europe (EC, 1993). Despite its positive effects on meat quality in comparison to the electrical stunning (Velarde *et al.*, 2001), it has been questioned on welfare grounds as CO₂ itself has been described as an aversive gas (Raj & Gregory, 1995).

Therefore, an adequate strategy is needed to avoid the negative effect of the *ante mortem* handling of pigs, especially those with the halothane gene. Some studies suggest that magnesium (Mg) or L-tryptophan (Trp) supplementation of the diet of pigs

can help to alleviate it and improve meat quality (D'Souza, *et al.*, 1998; Kietzmann & Jablosnki, 1985; Peeters *et al.*, 2005). Mg is a cofactor essential in many metabolic pathways (Stryer, 1988), it depresses the muscular activity due to its antagonism towards calcium and counteracts catecholamine effects in stressful situations by reducing levels of catecholamine in plasma (D'Souza, *et al.*, 1998; Kietzmann & Jablosnki, 1985). Some authors reported a positive effect of Mg supplementation on pig behaviour (Peeters *et al.*, 2005), water holding capacity of meat (WHC), pork colour and pork texture (D'Souza *et al.*, 1998; Caine *et al.*, 2000; Frederick *et al.*, 2006). Nevertheless, non conclusive results have been obtained when comparing the effect of Mg supplementation within carriers (Nn) and non-carriers (NN) of the Halothane gene (Apple *et al.*, 2000; Caine *et al.*, 2000; Panella-Riera *et al.* 2008).

Trp, as a precursor of serotonin (Fernstrom & Wurtman, 1971), has a potential sedative effect by promoting its synthesis in the brain (Leathwood, 1987). Contradictory results have been found with Trp supplementation with respect to meat quality: some authors claimed an increase of *post-mortem* muscle pH (Henry *et al.*, 1996) or a reduction of incidence of PSE meat (Adeola & Ball, 1992 and Henry *et al.*, 1996), while others reported no significant effect or even poorer meat quality (Guzik *et al.*, 2006; Li *et al.*, 2006).

A previous study reported the effect of magnesium carbonate (MgCO₃) and Trp on the welfare and on the carcass and meat quality of two halothane pig genotypes (NN and nn) under non-stressful conditions (Panella-Riera *et al.*, 2008). The aim of the present work was to evaluate the effect of dietary supplementation with magnesium and Trp on feed intake, mortality, animal behaviour and carcass and meat quality characteristics of NN and nn pigs under stressful *ante mortem* conditions.

1.2 Materials and methods

1.2.1 *Animals and diets*

Sixty-nine pure-breed entire male pigs were used in this study: 36 NN (24 Landrace and 12 Large White) and 33 nn (Pietrain), with an average live weight at slaughter of 108.5 ± 8.3kg. They were housed individually from the age of 90 to 150 days in pens with a space allowance of 4m², with visual and olfactory contact between each other. All were fed the same commercial diet (30.0% barley, 25.0% soy, 15.0% maize, 11.9% wheat

and a vitamin/mineral source) until 5 days before slaughter. The diet contained 87.9% of dry matter and 14.1MJ/kg of digestible energy on fresh matter.

Five days before slaughter three diet groups were established for each genotype: Control group, with no supplement; Mg&Trp group, control diet supplemented with 1.2g elemental Mg and 8g *L*-tryptophan per kg; and Trp group, control diet supplemented with 8g *L*-tryptophan per kg (Table IV.3-1). Diets were designed considering a minimum of feed intake of 2kg/day during the monitoring period (initial pig weight: 105.6 ± 7.0 kg). Individual feed intake was monitored for each pig during the 5 days prior to slaughter.

1.2.2 *Ante mortem treatment and slaughter procedure*

Two batches of 36 and 33 animals were slaughtered on two different days. The animals from each treatment were mixed in 6 different groups according to diet and genotype. The day before slaughter, pigs were fasted on-farm during 9h and then transported from the IRTA pig experimental station to the IRTA experimental abattoir for 1h on rough secondary roads. Lairage time for the first pig slaughtered was about 30 min and for the last one was 7h as they were slaughtered individually by exsanguination, after stunning with 90% CO₂. In each batch, the animals from the 6 groups were slaughtered alternatively (3 diets x 2 genotypes).

1.2.3 *Animal behaviour measurements*

Behaviour measurements were recorded along the corridor leading to the stunning system and in the CO₂ stunning unit following the procedure described by Panella-Riera *et al.* (2008). The raceway was 412 cm long and 60 cm wide, and it was lined with steel panels of 90 cm height to prevent the pig from seeing out of the raceway. It was divided into three different sections: raceway, ramp and entrance. Pigs were separated from the group, placed individually at the starting point of the raceway and allowed to cross the raceway to enter the CO₂ stunning crate. If after 1 min. the animal was reluctant to move, it was gently pushed into the next section. The following behavioural variables were scored in each section: presence of retreat attempts (when the pig backed away; Dodman, 1977), time to perform the first retreat attempt and time to cross each section (the time taken to cross the raceway, the entrance and the ramp).

The CO₂ stunning unit was a dip-lift system (Butina Aps, Copenhagen) that contained a crate (195 x 90 x 61 cm) with perforated floor to facilitate the distribution of the gas

inside. The CO₂ concentration at the bottom of the crate was 90% by volume in atmospheric air. The stunning procedure lasted 132s (23s descending, 23s ascending and 86s at the bottom of the crate). Behavioural variables were monitored during the exposure to the CO₂ 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 breath through a wide open mouth, which may involve stretching of the neck and which was considered to be an indicator of the onset of breathlessness; Velarde *et al.*, 2007), first gasp (time to perform the first gasp), presence of muscular excitation (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 and last muscular excitation (time to perform the first and last muscular excitation) 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). The presence of gagging, as low frequency inhalations with its neck towards its front legs and, sometimes, emitting sounds similar to snore (Gregory *et al.*, 1987), was also recorded. All recording times were synchronised with the time the pigs started to descend into the well.

1.2.4 *Chemical analysis of feed*

The feed was analysed for crude protein, crude fibre, ether extract, humidity and ash according to the Association of Official Analytical Chemists (AOAC, 1990). Magnesium and Trp content were also determined (AOAC, 1990; Table 1).

1.2.5 *Measurements of carcass and meat quality*

After sticking, and when carcasses were hoisted on the bleeding rail, fresh scratches on the skin were inspected and subjectively scored according to their severity (Barton-Gade *et al.* 1996).

Carcass measurements.

The carcass weight (kg) without flare fat, kidneys and diaphragm was determined individually. Carcass grading was carried out using the Fat-o-Meat'er grading probe (FOM) in the left side of the carcass and carcass lean percentage was estimated using the Spanish official equation (Gispert & Diestre, 1994). Visual conformation was determined following the photographic model for EC Pig Grading Grid (1= very good conformation to 4= very poor conformation).

Meat quality measurements.

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

Sampling.

Samples from the LT muscle were collected at 24h *pm*, at the 3/4 last rib level, to determine drip losses, following the reference method supported by OECD (Honikel, 1996).

Colour measurements were carried out at the last rib level with a Spectrophotometer Minolta 2002 on the CIELab space (CIE, 1976) using D65 illuminant and 10° standard observer. Before the measurement, a slice of 0,2 mm was removed, and the colour was measured after 5 min blooming.

After chilling, at 24h *pm* the LT muscle was removed from the left side of each carcass to take the samples for posterior analysis of intramuscular fat, cathepsin activities and texture. Samples of SM muscle were also taken. They were vacuum-packed in aluminium bags and frozen at -20°C until analysis.

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

Cathepsin activity measurements were performed in the LT and SM muscles at 24h *pm*. 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 and 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. Results are given in nIU (nmol.min⁻¹.g protein⁻¹).

Instrumental tenderness was determined on boneless LT muscle. Samples 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 of 75 °C. Afterwards, they were allowed to come to room temperature before a minimum of six pieces 3.0x1.5x1.5 cm was 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 (Møller, 1980).

1.2.6 *Statistical analyses*

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

Behavioural data.

Behavioural observations were count data, time intervals and binary data. As data were not normally distributed, the statistical analysis was performed using Proc GENMOD of SAS with binomial, multinomial, Poisson or negative binomial distributions (Cameron and Trivedi, 1998). A binomial distribution with logit model as link function was used to analyse the incidence of pigs showing voluntary movement, presence of retreat attempts and presence of muscular excitation. A multinomial distribution with cumlogit model as link function was used in retreat attempts in the raceway, ramp entrance and the whole way and the time to perform the first retreat attempt in the stunning system. A Poisson distribution with log model as a link function was used with the time to perform the 1st sniffing, time to start the 1st and the last muscular excitation, time to loss of posture and the time to the first gasp. Negative binomial distribution with a log model as a link function was used for the time to cross the raceway, time to perform the 1st retreat attempt in the raceway, and time to perform the 1st gagging in the stunning unit. The effects studied were diets (Control, Mg&Trp, Trp) and genotype (NN, nn) and the interaction between them. Significance was fixed at P<0.05 in all cases. The residual maximum likelihood was used as a method of estimation. In the case of Poisson and negative binomial distributions, the least square means of fixed effects (LSMEANS) were used when analysis of variance indicated differences. In the case of multinomial distribution, the estimate statements were used.

Carcass and meat quality data.

Feed intake, 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 adjusted with Tukey's honestly significant difference (HSD). Significance level was fixed at $P < 0.05$, although significances at $P < 0.1$ were taken into account as a tendency in some cases. The least square means of fixed effects (LSMEANS) were used when analysis of variance indicated differences.

Two types of statistical analysis were used, the first one included all pigs in the model and the second one included only pigs with feed intake $> 2\text{kg/day}$.

1.3 Results

1.3.1 *Feed intake*

Table IV.3-2 shows the feed intake according to diets and genotypes. No differences among diets ($P > 0.05$) were found during the 5-day treatment on the daily feed intake, whereas NN pigs showed higher feed intake ($P < 0.001$) than nn (3.26 vs 2.13kg/day).

As stated above, diet supplements were included in the feed considering a minimum feed intake of 2kg/day in order to meet the appropriate dose of Mg and/or Trp. Table IV.3-2 also shows the number of pigs with feed intake higher and lower than 2kg/day (adequate and inadequate supplement intake, respectively). Diet supplements had a significant effect ($P < 0.05$) in both cases in the number of pigs showing adequate and inadequate supplement intake. When considering pigs with adequate feed intake ($> 2\text{kg/day}$), no differences in the daily feed intake were observed. However, there were significantly more pigs from the Mg&Trp group with feed intake lower than 2kg/day (Mg&Trp: 10 pigs vs C: 2 and Trp:4 pigs). Regarding genotypes, NN had higher feed intake than nn ones, and these differences were also observed when pigs with adequate supplement intake were considered (feed intake $> 2\text{kg/day}$).

Sixteen pigs showed feed intake lower than 2kg/d, 5 of them with extremely low values ($< 0.5\text{kg/d}$). Nine of the 16 pigs with feed intake $< 2\text{kg/day}$ had also a negative growth rate, 4 from the Trp group and 10 from the Mg&Trp group (Figure IV.3-1).

1.3.2 *Mortality*

The mortality rate during the *ante mortem* treatment is shown in Table IV.3-3. When considering all the animals, 5 pigs died, all from the stress-sensitive genotype (nn). Regarding diets, none of the dead pigs belonged to the Trp group, 2 pigs were from the Mg&Trp group (8.7%) and 3 from the control one (13.0%). Among pigs with an adequate supplement intake (>2kg/day), 4.8% of Control and 7.7% of the Mg&Trp pigs died.

1.3.3 *Animal behaviour in the experimental abattoir*

Table IV.3-4 shows the behaviour of pigs in the corridor that led to the CO₂ stunning system, considering those pigs with a feed intake > 2kg/day. There were no differences in the proportion of pigs showing retreat attempts among diets (P>0.05) in the three sections of the raceway, and no differences were found when the whole way was considered. Genotype had a significant effect (P<0.05) on the proportion of pigs showing retreat attempts at the entrance of the CO₂ stunning system, as all the pigs that showed retreat attempts were NN pigs (NN: 20.6% ; nn: 0%).

The behaviour of the pigs was assessed in the CO₂ stunning unit during the descent into the pit. No differences were found between genotypes and among diets. The first behaviour exhibited was the time to perform the first retreat attempt (4.4 ± 0.3 s), followed by the sniffing (9.0 ± 0.4 s), and the gasping (22.1 ± 0.5 s). The loss of posture took place after 27.8 ± 0.8 s, and gagging took place at 47.8 ± 1.2 s. Muscular excitation began at 21.6 ± 0.4 s, finished at 35.5 ± 0.8 s and lasted 13.5 ± 0.8 s. No differences in the time to begin muscular excitation among diets were observed, but a significant effect was found in the duration (P<0.001): Trp pigs showed shorter muscular excitation than other diet groups (Trp: 9.9s vs C: 16.6 and Mg&Trp: 14.0s).

1.3.4 *Carcass and meat quality*

Diet supplements did not have any effect (P>0.05) on the carcass quality variables considering those pigs with feed intake > 2kg/day (Table IV.3-5). No differences among diets were found in any of them. Besides, genotype had a significant effect (p<0.001) on almost of the carcass variables (body weight, lean content, carcass length, fat depth at

last rib level and conformation,). Carcasses from nn pigs were shorter, leaner, showed higher conformation and had less fat depth at last rib level than NN ones.

Carcass skin lesions were scored according to the procedure described by Barton-Gade *et al.* (1996). In general, no differences were found in the number of pigs showing skin lesions (Table 6). More than 85% of pigs had skin lesions, with no significant differences among diets and between genotypes. Regarding the severity of skin lesions, significant differences were observed among diets. Mg&Trp pigs were more likely to show more intense skin lesions than C pigs.

Two different data sets depending on the feed intake were studied to analyse meat quality traits. The first one considered all pigs and the second one considered only pigs with feed intake higher than 2kg/day. Table IV.3-7 shows significance levels of meat quality traits considering both statistical analyses, according to diet or halothane gene effect. When considering all the pigs the diet tended to affect pH values at 24h in SM muscle (P= 0.010; C: 5.59, Mg&Trp: 5.79, Trp: 5.67), electrical conductivity in LT muscle (P= 0.09; C: 6.8, Mg&Trp: 7.0, Trp: 5.9mS), drip loss in LT muscle (P= 0.08; C: 8.7 , Mg&Trp: 7.6, Trp: 7.5%), and the L* value in LT muscle (P= 0.05; C: 54.08, Mg&Trp: 50.92, Trp: 51.22). However, when considering pigs with feed intake higher than 2kg/d, diet had only a significant effect on pH at 45 min in the LT muscle (C: 5.96, Mg&Trp: 5.96, Trp: 6.15; Table IV.3-6).

The same statistical analyses were performed on the incidence of PSE and DFD meat. Overall, in the LT muscle 34.9% of the carcasses showed PSE and 4.8% DFD traits. In the SM muscle, 28.6% of carcasses showed PSE and 11.1% showed DFD condition. Table 5 shows the percentage of pigs with feed intake > 2kg/day that showed PSE and DFD traits according to diets and genotypes. Diets did not affect the percentage of pigs with PSE traits in LT or SM muscles but affected the number of carcasses showing DFD traits in the SM muscle, with a higher percentage in the Trp group.

Genotype affected significantly most of the meat quality variables (Table IV.3-5 and Table IV.3-7). Similar significance levels were found either analysing data considering all pigs and considering only those with feed intake higher than 2kg/day, with the exception of the pH_{24h} in the SM muscle: considering all pigs, no differences were found (P=0.20) whereas a significant effect was observed with pigs eating more than 2kg feed/day, NN pigs showing higher pH₂₄ (P=0.02).

In comparison to NN pigs, nn ones showed lower pH at 45 min , lower pH at 24h, higher electrical conductivity and loin drip loss and higher value of L*, a* and b* variables than NN pigs (Table IV.3-5). A significant genotype effect was found in the percentage of pigs showing PSE traits ($P < 0.0001$), 75.0% of LT and 60.0% of the SM muscles from nn pigs were PSE, while none of the NN pigs showed PSE traits (Table IV.3-5).

The activity of cathepsin B in LT muscle tended to be lower in the Mg&Trp and Trp groups than in the C group ($P = 0.07$; Table IV.3-8). No significant effect was observed among diets in the activity of cathepsin B+L enzymes. Genotype affected cathepsin B+L activity in LT and SM muscles ($P < 0.05$): nn pigs showed higher enzymatic activity than NN pigs; but no effect was found in cathepsin B enzymatic activity (Table IV.3-8). Texture measurements were performed on non-aged meat (frozen at 24h *pm*). The values of shear force (Warner-Bratzler test) neither depended on the diet supplements nor on the pig genotype ($P > 0.1$).

1.4 Discussion

1.4.1 *Feed intake and mortality*

The transport between the farm and the experimental abattoir was performed under stressful conditions. As a result 5 pigs died, all of them being halothane positive (nn). Mortality during transportation to slaughter plant reflects the high levels of stress suffered by the pigs during the journey. A genetic susceptibility, as well as some inadequate procedures when loading pigs may add sufficient stress to poor transport conditions to result in deaths (Warris, 1998) and, as stated by Gispert *et al.* (1996), high mortality rates may lead to important economic losses. Our results are consistent with the fact that the halothane gene is related to a high sensitivity to stress (Fàbrega *et al.*, 2002) and that if nn pigs are submitted to minimal stressful conditions, losses before slaughter can be avoided (Panella-Riera *et al.*, 2008). In accordance with these results, Geers *et al.* (1994) and Pérez *et al.* (2002) also found a greater effect of the handling and transport before slaughter on nn pigs than on NN pigs.

In studies assessing the effect of a diet supplement, it is very important to consider the individual feed intake especially when the supplement is provided through the feed. In the present study, the feed intake was measured individually during the 5-day treatment.

Supplemented feeds were designed considering a minimum feed intake of 2kg/day for pigs with a live weight of 105.7 ± 7.0 kg (Fàbrega *et al.*, 2003). Therefore, pigs with feed intake lower than 2kg/day did not ingest enough supplements, Mg and/or Trp. When considering pigs with the desired supplement intake, only 2 died, one from the control group and the other one from the Mg&Trp group. Further research should be carried out to ascertain whether there is any relationship between the mortality of pigs and the Trp supplementation as it has been suggested that the sedative effect of this amino acid could counteract the negative effect of a stressful pre-slaughter treatment (Kietzmann & Jablonski, 1985).

One of the hypotheses of the present study was that after removing pigs with inadequate supplement intake, diet effect on pigs' behaviour and meat quality may vary. Therefore, data were analysed firstly, considering all pigs, and secondly including only pigs with an adequate supplement intake (> 2kg/day).

No differences among diets were found in the average feed intake, probably due to the wide variability in feed intake within treatments. However, differences were observed in the number of pigs with feed intake lower than 2kg/day. In the Mg&Trp group there were significantly more pigs with inadequate supplement intake than in Control group. All the pigs that lost weight during the treatment had a feed intake lower than 2kg/d. Among them, two different groups can be distinguished. The first one that lost between 0.4 and 0.6kg/day had a feed intake lower than 0.5kg during the treatment, indicating that they scarcely ate (Figure IV.3-1). All these pigs belonged to the supplemented diets (Mg&Trp and Trp groups) so it could be suggested that, apart from the effect of changing the diet, pigs did not like the new feed. The second group, all from the Mg&Trp group, includes pigs with feed intake from 1 to 2kg/day that lost weight anyway. This may be due to the potential laxative/purgative effect associated with magnesium sulphate (Frederick *et al.*, 2004; O'Quinn *et al.*, 2000), as diarrhoea was observed in Mg&Trp pens. D'Souza *et al.* (1999) suggested that although aspartate, chloride and sulphate salts of Mg were equally effective in reducing the incidence of PSE pork, MgSO₄ could be the best choice because magnesium aspartate was an expensive source of Mg and the chloride salt had chemical properties that make its inclusion in the feed difficult. Hamilton *et al.* (2002) also concluded that MgSO₄ produced the greatest improvement in meat quality traits; more than proteinate and propionate sources. However, none of these studies reported a potential laxative effect

of the MgSO₄ affecting muscle glycogen stores and therefore the *post-mortem* energy metabolism.

To our knowledge, few studies have reported the effect of 5-day Mg and/or Trp supplementation on feed intake and none of them found any effect of the inclusion of supplements of Mg or Trp in the diet (Caine *et al.*, 2000; Peeters *et al.*, 2005). However, most of them reported an effect of Mg supplements on meat colour (Caine *et al.*, 2000; D'Souza *et al.*, 1998; Hamilton *et al.*, 2002) and water holding capacity (D'Souza *et al.*, 1998, 1999; Hamilton *et al.*, 2002; Schaefer *et al.*, 1993).

When Frederick *et al.* (2004) studied the effect of Mg supplementation through drinking water, no differences in feed intake and water disappearance were found among different Mg concentrations.

On the other hand, the relationship between Trp consumption and the feed intake was extensively discussed by Le Floc'h & Sève (2007). While Trp deficiency was related to a reduction of appetite and feed intake for finishing pigs (Henry *et al.*, 1992), an excess of 4% of Trp in the diet was also reported to decrease the feed intake in finishing pigs (Chung *et al.*, 1991).

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

The first behaviour that pigs exhibited in the cage when they were exposed to the gas was retreat attempts, followed by sniffs and gasp indicating the onset of breathlessness according to Raj (1999) and Gregory *et al.* (1987). Almost simultaneously, pigs showed muscular excitation from 21.6 ± 0.4 to 35.3 ± 0.8 s lasting an average time of 13.5 ± 0.8 s. Similar results were found by Rodriguez *et al.* (2008), who found muscular excitation appeared between 19 and 39s.

Our results indicated that genotype tended to have an effect on the duration of muscular excitation, lasting 14.6 ± 1.14 s for the nn pigs and 12.8 ± 1.00 s for the NN ones. Diet had a significant effect, as the muscular excitation period in Trp-group was shorter than in C and Mg&Trp ones. In contrast to these results, no differences between genotypes (NN vs nn) and among supplemented-diets (Control, Mg, Trp) were found in the previous experiment carried out under conditions of minimal stress during transport (Panella-Riera *et al.*, 2008).

Rodriguez *et al.* (2008) suggest that the onset of muscular excitation is likely to occur when pigs are still conscious. On the other hand, the loss of posture during the exposure to gas has been considered by some authors to be the first behavioural indicator of the onset of unconsciousness (Raj & Gregory, 1996). In the present study, pigs lost their posture at 27.8 ± 0.8 s after the beginning of the stunning procedure, taking place during the presence of muscular excitation. In fact, for 13.2% of animals the muscular excitation appeared earlier than the loss of posture, for 5.7% appeared at the same time, and for 81.1% the loss of posture took place before the appearance of muscular excitation. So it is not clear if these muscular excitations are escape attempts, as described by Raj & Gregory (1996) or involuntary convulsions, as described by Forslid (1987).

In general, no differences were found among diets and between genotypes on animal behaviour. However, in agreement with Raj (2004) and Velarde *et al.*, (2007), our results indicated that the induction of unconsciousness with carbon dioxide, by using a dip-lift system, could be distressing to animals and thus compromise the welfare of pigs. Therefore, the meat industry should be encouraged to consider potential alternatives (Raj, 2004; Velarde *et al.*, 2007). The role of Trp in the muscular excitation of animals inside the system needs further investigation.

1.4.3 *Carcass and meat quality*

Carcass quality was positively affected by halothane genotype, with the nn pigs having leaner carcasses and higher conformation (lower score) than the NN ones. These are considered relevant traits for ham production in Spain and for that reason halothane genotype is still desired by many producers and the meat industry in Europe despite knowing the detrimental effects on pork quality. The negative effect of the halothane gene to meat quality traits has been extensively studied (e.g. Jensen & Barton Gade, 1985; Channon *et al.*, 2000; Gispert *et al.*, 2000; Guàrdia *et al.*, 2004). Accordingly, in the present study the nn pigs showed lower pH values and water holding capacity (higher drip loss and electrical conductivity) and paler meat (L^* value) than the NN ones. Similar results were also obtained in the previous study under non-stressful *ante mortem* conditions (Panella-Riera *et al.*, 2008).

Supplementation with Mg and/or Trp had no effect ($P>0.05$) on midline back fat measurements, last rib fat depth and lean content (Table IV.3-5). These results are

consistent with other studies that did not find any effect of Mg supplementation on fat and muscle measurements of pig carcasses after a 5-day treatment (Schaefer *et al.*, 1993; D'Souza *et al.*, 1998, 1999).

Carcass quality is also affected by the presence of skin lesions as this may determine the commercial application (Barton-Gade *et al.*, 1996). In the present experiment, no differences were observed in the number of pigs showing skin lesions, as most of them had lesions on the skin. This may be explained by the fact that pigs tried to establish hierarchy at the lairage pens as until then they did not have the chance to do it. However, differences in the severity of these lesions were observed among diets. Mg&Trp pigs were more likely to have more intense skin lesions than C ones. This may be related to the fact that these pigs may be hungry due to the laxative effect of magnesium sulphate. There is also some evidence that fasted pigs tend to fight more aggressively and for a longer time than fed pigs (Fernandez *et al.*, 1995). According to Barton-Gade *et al.* (1996), a score of between 3 and 4 is unacceptable for most of the commercial applications, and even a score of 2 should be also considered unacceptable when carcasses are used for sensitive productions. In the case of Mg&Trp diet, more than 83.3% of the carcasses showed a score higher than 3, and 100% of the carcasses had a score of ≥ 2 . Therefore, in the present experiment 83.3% of carcasses would be unacceptable for most commercial applications.

Meat quality traits were also studied. When taking into account all pigs, diet tended to affect pH at 24h in SM muscle, electrical conductivity and drip loss in LT muscle and showed a statistically significant effect on colour (Table IV.3-7). As magnesium sulphate has been associated with a laxative effect, pigs may have had depleted muscle glycogen stores and, therefore it may produce meat with higher ultimate pH, darker colour and higher water holding capacity (Lambooij, 2007). These differences were not found when the data were analysed with pigs with adequate supplement intake, as diets only had an effect on pH measured at 45 min at LT muscle.

As shown in Table IV.3-5, high incidence of pigs still showed PSE traits ($\text{pH}_{45} \leq 5.8$) after removing those with feed intake lower than 2kg/day. A total of 16 pigs were removed from the statistical analysis for their inadequate supplement intake, most of them showing PSE traits (8 in the LT and 6 in the SM muscle), all of them nn pigs. These results confirm that the pre-slaughter treatment was indeed under stressful conditions, and also confirm the relationship between the halothane gene and the

incidence of PSE meat. Besides, two pigs from the removed ones were classified as DFD ($\text{pH}_{24\text{h}} \geq 6.0$) in the SM muscle which was the expected result as DFD meat occurs when the animals are fatigued and the glycogen energy store is exhausted at slaughter (Lambooi, 2007).

Despite the higher incidence of PSE, it is surprising the higher incidence of DFD meat among pigs with an adequate supplement intake. Pigs from the Trp group showed higher incidence of DFD in the SM muscle than C and Mg&Trp, indicating that the supplementation of this amino acid could not completely avoid the depletion of the glycogen under a long stressful *ante-mortem* treatment, questioning the initial hypothesis. Further research is needed to ascertain whether the increase of the pH is due to the Trp supplement or the long stressful *ante-mortem* treatment. Henry *et al.* (1996) concluded that *post-mortem* muscle pH increased (both, pH₄₅ and pH_u) in response to a long term Trp supplementation.

In agreement with the previous study (Panella-Riera *et al.*, 2008), cathepsin activities were influenced by the genotype but not by the diet (Table 8): nn pigs showed higher activity than NN ones. Genetic type is known to affect cathepsin activity (Hernández *et al.*, 2004; Plastow *et al.*, 2005).

No differences were found between the two genotypes with instrumental measurements (Shear force test). However the panel test carried out within the same experiment found that the meat from nn pigs was harder than NN ones (Panella *et al.*, 2005). Controversial results have been published. De Smet *et al.*, (1996) did not find any differences with instrumental measurements between genotypes. However, Moelich *et al.* (2003) and Murray *et al.* (1989) reported higher shear force values in meat from nn pigs than in meat from NN pigs.

1.5 Conclusions and implications

When studying the use of diet supplements, the individual measurement of feed intake should be a requirement as it allows the detection of pigs with inadequate supplement intake, even when the average feed intake is the expected value.

Potential side effects of the diet supplements should be taken into account as they may lead to misleading conclusions. The inclusion of a mixture of magnesium sulphate and tryptophan in the diet did not show any advantage due to the laxative effect of

magnesium sulphate, showing lower feed intake. The Mg&Trp group showed higher probability of having severe skin lesions in comparison to Control and Trp groups. From the results of this study the utilization of a mixture of magnesium sulphate and tryptophan is not recommended.

Trp reduced the duration of muscular excitation when animals were stunned with high concentrations of CO₂, and pigs showed the same probability as control pigs of having severe skin lesions. An increase of pH at 45 min *pm* in the LT muscle and the percentage of pigs showing DFD traits in the SM muscle were observed. Further research is needed to ascertain whether Trp can prevent the mortality related to high levels of stress during the pre-slaughter period, and whether the increase of pH is due to the Trp supplement or to the stressful *ante-mortem* treatment.

A new strategy, such as the combination of Trp with other compounds or other sources of magnesium (excluding magnesium sulphate) should be considered for further studies, with the aim of reducing stress before slaughter and being able to reduce the negative effect of the use of the halothane gene.

1.6 References

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1.7 Figures

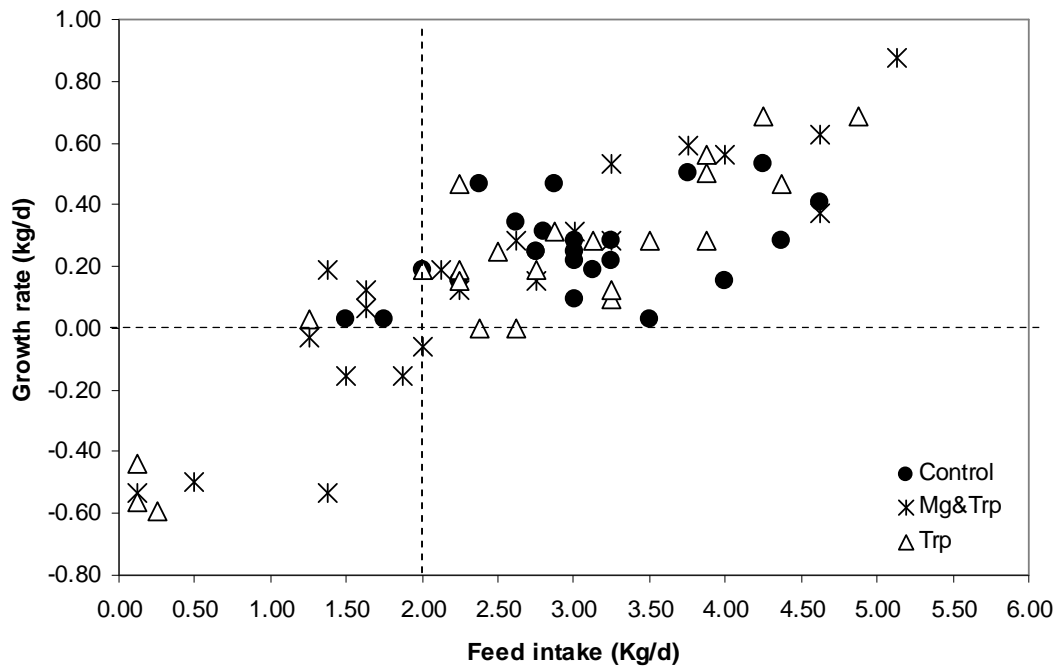


Figure IV.3-1. Relationship between feed intake (kg/day) and growth rate (kg/day) among diets.

1.8 Tables

Taula IV.3-1. Feed composition of experimental and control diets (%).

	Diets		
	C	Mg&Trp	Trp
Humidity (%)	11.80	11.47	11.80
Ash (%)	6.79	6.78	6.87
Crude fiber (%)	3.12	3.40	3.24
Crude protein (%)	16.80	18.54	18.4
Ether extract (%)	6.84	6.30	6.19
Magnesium (%)	0.21	0.40	0.20
Tryptophan (%)	0.21	0.70	0.77

C: Control Diet; Mg&Trp: Control diet with Mg and Trp supplementation; Trp: Control diet with Trp supplementation.

Taula IV.3-2. Number of pigs with feed intake higher and lower than 2kg/d, and feed intake (LSM¹) according to the three diets and two genotypes.

	Diet				Genotype		
	C	Mg&Trp	Trp	Sig	NN	nn	Sig
Number of pigs ²							
In the experiment	23	23	23		36	33	
With feed intake \geq 2kg/d	21 (91.3)	13 (56.5)	19 (82.6)		30 (83.3)	23 (69.7)	
With feed intake < 2kg/d	2 (8.7)	10 (43.5)	4 (17.4)		6 (16.7)	10 (30.3)	
	Sig. ³ a	b	ab	**	-	-	ns
Feed intake ¹							
Total feed intake (kg/d)	3.00 (0.19)	2.40 (0.19)	2.67 (0.19)	ns	3.26 (0.16)	2.13 (0.17)	***
Pigs with feed intake \geq 2kg/d	3.09 (0.13)	3.20 (0.17)	3.13 (0.14)	ns	3.07 (0.11)	2.58 (0.13)	***
Pigs with feed intake < 2kg/d	1.69 ^a (0.41)	1.26 ^a (0.17)	0.44 ^b (0.27)	*	1.21 (0.26)	1.06 (0.19)	ns

C: Control Diet; Mg&Trp: Control diet with Mg and Trp supplementation; Trp: Control diet with Trp supplementation; ns: P>0.1; *: P<0.05; **: P<0.01 and ***: P<0.001.

¹ LSM: Least square means and standard error within brackets.

² Number of pigs and percentage within brackets.

³ Columns with different letter were significantly different at a level of P<0.05. The probability of showing feed intake < 2kg/day was tested with a GENMOD procedure.

Taula IV.3-3. Mortality of pigs during the pre-slaughter period according to the three diets and two genotypes.

	Diet				Genotype		
	C	Mg&Trp	Trp	Sig	NN	nn	Sig
Mortality considering all pigs included in the experiment							
n	23	23	23		36	33	
deaths	3	2	0	ns	0	5	*
(%)	13.0	8.7	0.0		0.0	15.2	
Mortality between pigs with feed intake > 2kg/d							
n	21	13	19		30	23	
deaths	1	1	0	ns	0	2	ns
(%)	4.8	7.7	0.0		0.0	8.7	

C: Control Diet; Mg&Trp: Control diet with Mg and Trp supplementation; Trp: Control diet with Trp supplementation; ns: P>0.1 and *: P<0.05.

Taula IV.3-4. Time taken to cross the raceway¹, Percentage of pigs showing retreat attempts and time to perform the first retreat attempt according to the three diets and two genotypes. Pigs with feed intakes >2kg/day are considered.

	Diet				Genotype			
	n	C	Mg&Trp	Trp	Sig	NN	nn	Sig
Time to cross (s)								
the raceway		18.4 (4.8)	30.7 (12.0)	32.3 (7.4)	ns	26.1 (5.9)	26.7 (6.5)	ns
the ramp		41.2 (7.8)	50.6 (10.1)	51.3 (10.9)	ns	49.7 (7.6)	42.7 (7.6)	ns
the entrance		38.6 (8.2)	26.8 (9.6)	42.2 (7.2)	ns	43.2 (6.8)	27.3 (5.2)	*
all the way		98.2 (10.7)	108.1 (25.2)	125.8 (17.0)	ns	119.0 (13.5)	96.7 (11.5)	ns
Pigs showing retreat attempts (%)								
in the raceway		4.8	15.4	21.1	ns	10.0	17.4	ns
in the ramp		47.6	38.5	36.8	ns	50.0	30.4	ns
in the entrance		14.3	7.7	10.5	ns	20.6	0.0	*
all the way		57.1	46.2	63.2	ns	66.7	43.5	ns

C: Control Diet; Mg&Trp: Control diet with Mg and Trp supplementation; Trp: Control diet with Trp supplementation; ns: P>0.1 and *: P<0.05.

¹Means and standard error

Taula IV.3-5. Carcass and meat quality traits of diet and genotype effects, measured considering pigs with feed intake higher than 2kg /day.

	n	Diet ¹			Sig.	Genotype ¹		
		C	Mg&Trp	Trp		NN	nn	Sig.
		21	13	19		30	23	
<u>Carcass traits</u>								
Body weight (kg)		109.5 (1.8)	109.8 (2.3)	110.4 (1.8)	ns	113.6 (1.4)	106.2 (1.8)	**
Carcass weight (kg)		85.1 (1.4)	84.2 (1.8)	85.4 (1.4)	ns	85.5 (1.4)	84.2 (1.4)	ns
Lean content (%)		57.5 (0.63)	56.6 (0.79)	56.6 (0.62)	ns	52.7 (0.50)	61.1 (0.62)	***
Carcass length (cm)		81.6 (0.9)	81.3 (1.1)	82.0 (0.9)	ns	85.8 (0.7)	77.5 (0.9)	***
Fat depth at last rib level (FOM; mm)		12.9 (0.6)	13.4 (0.8)	13.5 (0.6)	ns	16.6 (0.5)	10.0 (0.6)	***
Conformation		2.0 (0.1)	2.1 (0.1)	2.0 (0.1)		2.9 (0.1)	1.2 (0.1)	
<u>Meat quality parameters</u>								
pH at 45 min								
LT		5.96 ^b (0.06)	5.96 ^b (0.07)	6.15 ^a (0.06)	*	6.47 (0.05)	5.58 (0.06)	***
SM		6.09 (0.06)	6.11 (0.07)	6.21 (0.05)	ns	6.47 (0.04)	5.79 (0.05)	***
pH at 24h								
LT		5.47 (0.05)	5.52 (0.06)	5.59 (0.05)	ns	5.60 (0.04)	5.46 (0.05)	*
SM		5.59 (0.06)	5.71 (0.07)	5.71 (0.06)	ns	5.75 (0.05)	5.58 (0.06)	*
Electrical Conductivity 24h (mS)								
LT		7.0 (0.4)	7.1 (0.5)	6.0 (0.4)	†	4.2 (0.3)	9.3 (0.4)	***
SM		8.4 (0.5)	7.3 (0.6)	7.0 (0.5)	ns	5.2 (0.4)	9.9 (0.5)	***
Loin drip loss (%)		8.7 (0.5)	8.0 (0.6)	7.4 (0.5)		5.1 (0.4)	11.0 (0.5)	***
Colour- L*		54.2 (1.1)	51.9 (1.4)	51.1 (1.1)	ns	50.0 (0.9)	54.8 (1.1)	***
Incidence of PSE (pH ₄₅ ≤ 5.8;%)								
LT		31.6	33.3	26.3	ns	0.0	75.0	***
SM		21.1	25.0	26.3	ns	0.0	60.0	***
Incidence of DFD (pH _u ≥ 6.0;%)								
LT		0.0	8.3	10.5	ns	6.7	5.0	ns
SM		0.0 ^b	8.3 ^b	21.1 ^a	*	13.3	5.0	ns

¹ Least square means and standard error within brackets.

C: Control Diet; Mg&Trp: Control diet with Mg and Trp supplementation; Trp: Control diet with Trp supplementation; SM: *Semimembranosus* muscle; LT: *Longissimus thoracis* muscle; PSE: Pale, Soft and Exudative; DFD: Dark, Firm and Dry; ns: P>0.1; †: P<0.1; *: P<0.05; **: P<0.01 and ***: P<0.001.

Taula IV.3-6. Percentage of carcasses with skin lesions according to the three diets and two genotypes. Pigs with feed intake > 2kg/day are considered.

	Diet ¹				Genotype ¹		
	C	Mg&Trp	Trp	Sig	NN	nn	Sig
n	21	13	19		30	23	
Skin lesions (%)	85.7	100	94.7	ns	93.3	91.3	ns
Severity of skin lesions							
No skin damage (1)	16.7	0.0	5.6		6.9	10.5	
Slight skin damage (2)	55.6	16.7	33.3		37.9	36.8	
Skin damage affecting quality (3)	22.2	50.0	50.0		37.9	42.1	
Severe skin damage (4)	5.6	33.3	11.1		17.2	10.5	
Sig. ¹	b	a	b	**	-	-	ns

C: Control Diet; Mg&Trp: Control diet with Mg and Trp supplementation; Trp: Control diet with Trp supplementation; ns: P>0.05; **: P<0.01.

¹ Columns with different letter were significantly different at a level of P<0.05. The probability of showing more intense skin lesions (from 1 to 4) was tested with a GENMOD procedure. Diet effect: P= 0.0025; Genotype effect: P=0.5769.

Taula IV.3-7. Significance levels of meat quality variables of diet and genotype effects, measured considering all pigs and those pigs with feed intake higher than 2kg /day.

		Diet effect		Halothane effect	
		All pigs	Feed intake > 2kg/d	All pigs	Feed intake > 2kg/d
	n	64	50	64	50
pH at 45 min	LT	0.34	0.05	<.0001	<.0001
	SM	0.64	0.26	<.0001	<.0001
pH at 24h	LT	0.21	0.24	0.0070	0.03
	SM	0.10	0.25	0.21	0.02
Electrical Conductivity at 24h (mS)	LT	0.09	0.11	<.0001	<.0001
	SM	0.47	0.11	<.0001	<.0001
Loin drip loss (%)	LT	0.07	0.13	<.0001	<.0001
Colour	L*	0.05	0.13	<.0001	0.001
	a*	0.10	0.37	<.0001	<.0001
	b*	0.42	0.42	<.0001	<.0001

SM: *Semimembranosus* muscle; LT: *Longissimus thoracis* muscle.
P values lower than 0.1 are showed in bold.

Table IV.3-8. Enzymatic activity variables ¹ (cathepsin B and cathepsin B+L) and instrumental texture variables ¹ after Warner-Bratzler Shear force test, among diets and halothane genotype. Pigs with feed intake > 2kg/day are considered.

	Diet				Genotype		
	C	Mg&Trp	Trp	Sig	NN	nn	Sig
n	21	13	19		30	23	
<i>Enzymatic activity</i> ²							
CAT B (nIU)							
LT	32.3 (3.1)	26.3 (3.9)	22.1 (3.0)	ns	24.2 (2.4)	30.5 (3.0)	ns
SM	79.8 (6.6)	72.9 (8.3)	71.1 (6.5)	ns	71.4 (5.2)	77.8 (6.5)	ns
CAT B+L (nIU)							
LT	161.9 (13.5)	159.0 (17.0)	151.4 (17.0)	ns	135.2 (10.7)	179.6 (13.3)	**
SM	251.6 (16.1)	255.7 (20.2)	281.1 (15.9)	ns	243.4 (12.7)	282.2 (15.8)	*
<i>Instrumental texture</i>							
Shear Force (N)	51.2 (2.2)	50.4 (2.7)	48.2 (2.3)	ns	50.5 (1.8)	49.4 (2.2)	ns
Initial Yield Force (Ncm)	72.8 (3.3)	72.0 (4.1)	70.4 (3.5)	ns	69.3 (2.7)	74.1 (3.3)	ns
Total Work (kg.s/cm ²)	6.7 (0.3)	6.1 (0.3)	6.1 (0.3)	ns	6.4 (0.2)	6.2 (0.3)	ns

C: Control Diet; Mg&Trp: Control diet with Mg and Trp supplementation; Trp: Control diet with Trp supplementation; LT: *Longissimus thoracis* muscle; SM: *Semimembranosus* muscle; CAT B: cathepsin B activity; CAT B+L: cathepsin B+L activity; ns: P>0.1 *: P<0.05 and **: P<0.01.

¹ Least square means and standard error within brackets.

² Results are given in nIU (nmol.min⁻¹.g proteine⁻¹)

