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The effect of stress immediately prior to stunning on proglycogen, macroglycogen, lactate and meat quality traits in different pig breeds

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ABSTRACT

The aim of the study was to evaluate the levels of lactate, pro-, macro-, and total glycogen in two different muscles, and meat quality traits (pH, colour, drip loss, cooking loss and shear force) across three different pig breeds, in the presence and absence of physical short-term pre-slaughter stress. Twenty-eight halothane-free pigs of the Italian Large White, Duroc and Pietrain breeds were subjected to different kinds of pre-slaughter handling: rough (RPH) or gentle (GPH). Before stunning, RPH group of pigs were subjected to a fast driving supported by the use of electric prods, whereas GPH group pigs were driven slowly without electric prods. Handling procedure influenced the contents of macroglycogen, lactate and pH at 45-min post-mortem with no effect on meat quality traits at 24 and 72 h post-mortem. Irrespective of the handling procedure, breed significantly affected L* and b* colour coordinates, Pietrain pigs showing slightly paler meat.

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Breeds; glycogen forms; meat quality traits; pigs; handling procedures

Introduction

It is well-known that the store of muscular glycogen at slaughter plays a decisive role on the successive rate and extent of post-mortem glycogen metabolism in pig muscles. Research on glycogen synthesis and storage has led to a renewed interest on the two constituent fractions, i.e. proglycogen and macroglycogen (Pösö and Puolanne 2005). The proglycogen fraction is acid-insoluble, has a molecular weight (MW) of ~400 kDa, and a low ratio of glucose units to protein; the macroglycogen fraction is acid-soluble, has a MW of ~104 kDa, and a high ratio of carbohydrate-to-protein content. These two forms are metabolised with different priorities under aerobic and anaerobic conditions (Graham et al. 2001).

Short-term stress, immediately before stunning increases the decline rate of pH and temperature in early post-mortem, with a consequent detrimental effect on several pork meat quality traits (Rosenvold and Andersen 2003). In this situation, the muscular glycogen store is subjected to a rapid degradation, both *in vivo* and early post-mortem, but the role of the glycogen forms in accelerating post-mortem glycolysis and their relationships with pork meat quality are not well-known yet. The purpose of the present study was to investigate the effect of short-term physical stress prior to stunning on muscular proglycogen, macroglycogen, and lactate and on several meat quality traits in three different pure pig breeds.

Materials and methods

The experimental protocol complied with the rules approved by the Ethic Committee of University of Bologna. The physical stress conditions applied before stunning were carried out in accordance with the Council Regulation (EC) procedure number 1099/2009 (EC 2009) and it conforms to the provisions of the Declaration of Helsinki (WMA 2008).

Animals and experimental design

A total of 28 purebred, unrelated, castrated male pigs were used; these included 10 Italian Large White (ILW), 10 Italian Duroc (IDU), and 8 Pietrain (PI) pigs. These pigs were clinically healthy and non-carriers of the recessive T allele (or n allele) of the g.1843C > T polymorphism of the ryanodine receptor-1 (*RYR1* or

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halothane) gene (Fujii et al. 1991). Piglets were collected at ~30 kg live weight from the experimental research farm of our Department. They were randomly distributed amongst six pens. They were fed *ad libitum* for 115 days using a commercial feed pellet, containing 16% crude protein (0.8% lysine), and 14.1 MJ/kg digestible energy. All pigs had *ad libitum* access to water via a nipple drinker. At the end of the 115-day growing period, live weight [mean ± SD (standard deviation)] were 114.0 ± 20.05 kg for ILW, 123.0 ± 12.57 kg for IDU, and 115.2 ± 9.24 kg for PI. All pigs were slaughtered in a single day at the same abattoir. Before slaughter, subjects of each breed were allocated randomly into two groups (five ILW, five IDU, and four PI per group): gentle pre-slaughter handling (GPH, or non-stressed) and rough pre-slaughter handling (RPH, or stressed).

Pre-slaughter condition

The farm was located 127 km from the abattoir; transport time was ~2 h, and the pigs were slaughtered immediately upon their arrival. The physical stress treatment for pigs of the RPH group began ~5 min before slaughter. These pigs were subjected to a fast driving, consisting of a 25 m run along the raceway between the resting pens and the stunning area of the abattoir. Run was maintained by the use of electric prods, using shocks no longer than one second. Pigs in the GPH group were driven slowly for the same distance, without the use of electric prods. All animals were stunned by electronarcosis (V 220, Amp 1.3) before exsanguination.

Sampling and meat quality analyses

At 45-min post-mortem, pH (pH_{45}) was determined in the *Longissimus thoracis* (LT) at the level of last rib, and in the *Semimembranosus* m. (SM) both on the left side of carcase using a pH-metre equipped with a glass electrode (model 5232; Crison Instruments SA, Modena, Italy). After 24 h at a temperature of 0–4 °C, pH was measured again (pH_{24}) in the LT muscle, in the same position. Due to dissection of hams before 24 h, it was not possible to measure pH_{24} of SM. In conjunction with the pH_{45} measurement, ~50 g of muscle tissues from LT and SM were collected from each animal, immediately frozen, and stored in liquid nitrogen for subsequent analysis of proglycogen, macroglycogen (Adamo and Graham 1998) and lactate (kit 735 Sigma, Schnelldorf, Germany).

Meat quality parameters (instrumental colour, cooking loss, drip loss, and shear force) were evaluated on ~50 g of LT samples collected at the level of the last rib from the left side of the carcase. Colour (CIE-L*a*b* system) was measured at 24 and 72 h after slaughtering (CR310 Minolta Chroma Meter with D65 light source; Osaka, Japan) after 30 min of blooming. Drip loss was determined 48 h after slaughter according to Honikel (1998) on samples of 30 g weighed and suspended in inflated bags, at 0–4 °C, for 48 h. Drip loss was expressed as a percentage according to the following formula: [(initial weight − final weight)/initial weight] × 100. To measure cooking loss, the same samples were cooked in a water bath until the sample temperature reached 75 °C (Honikel 1998). Cooking loss was calculated as the difference between the sample weight before and after cooking, expressed as percentage. Shear force was determined after cooking of the same samples which were allowed to stand to reach room temperature (~15 °C), then cut into 1 × 1 × 2 cm pieces, and placed into a Universal Testing machine (Model 1011, Instron Corp., Canton, MA) equipped by a Warner–Bratzler shear head with a cross speed of 200 mm/min.

Statistical analyses

Data were analysed with PROC MIXED of SAS version 9.4 (SAS Institute, Inc., Cary, NC). Data collected at 45-min post-mortem were processed with a model including the fixed effects of pre-slaughter handling group (RPH and GPH), muscle (LT and SM), breed (ILW, IDU, and PI), and their interactions as fixed factors. The breed and handling group factors were tested against animals within breed and group. The residual mean square was used as the error term for other effects. The same model without the factor muscle was used for the measures collected on LT only. When the interactions were significant, the means were compared by Tukey–Kramer test at a significance level of $p = .05$. Carcass weight was initially used as covariate, but was not significant and then was removed from the model.

Results and discussion

Based on molecular diagnostic test, all pigs were halothane-free (genotype homozygous g.1843CC of *RYR1*, Fujii et al. 1991). The effects of pre-slaughter handling, muscle and breed on pH_{45}, lactate, pro-, macro-, and total glycogen are shown in Table 1. The group of pigs rough handled (RPH) showed pH_{45} values significantly lower ($p = .016$) and lactate content significantly higher ($p = .008$), confirming the effect of this practice in accelerating the glycolytic process

Table 1. Effect of pre-slaughter handling, muscle and breed on pH, lactate, proglycogen, macroglycogen, and total glycogen measured at 45-min post-mortem[c].

	Pre-slaughter handling, Pre-s.		Muscle		Breed			*p* value			
Variable	Gentled	Rough	LT	SM	ILW	IDU	PI	Pre-s.	Muscle	Breed	R-MSE
pH_{45}	6.45[a]	6.28[b]	6.37	6.35	6.35	6.35	6.28	.016	ns	ns	0.19
Lactate, µmol/g	34.83[b]	45.01[a]	43.74[a]	36.10[b]	42.64	35.73	41.39	.008	.004	ns	10.99
Proglycogen, µmol/g	71.62	63.59	64.68	70.90	74.03	66.23	63.10	ns	ns	ns	4.69
Macroglycogen, µmol/g[c]	13.97[a]	8.86[b]	9.62[b]	13.22[a]	12.93	11.91	9.42	.004	<.001	ns	24.87
Total glycogen, µmol/g	85.60	72.82	74.27	84.14	86.97	78.14	72.52	ns	ns	ns	25.50

ns: not significant; LT: *Longissimus thoracis* m.; SM.: *Semimembranosus* m.; ILW: Italian Large White; IDU: Italian Duroc; PI: Pietrain; R-MSE: root mean square error.
[a,b]Different letters in the same row denote significant ($p < .05$) differences.
[c]Significant interaction on macroglycogen between pre-slaughter handling and muscle, and between muscle and breed are shown in Figure 1.

(Henckel et al. 2002; Hambrecht et al. 2005; Dokmanović et al. 2014). A significant difference in lactate content was found between LT and SM muscles ($p = .004$). The lower lactate levels in SM may be due to the lower activity in this muscle of glycogen debranching enzyme, which is essential to control the rate of glycogenolysis and glycolysis (Kylä-Puhju et al. 2005). The levels of pro- and total glycogen were not affected by handling before slaughter, muscle and breed ($p > .05$). There was not a significant pre-slaughter handling effect on proglycogen ($p > .05$) probably because it is largely degraded anaerobically (Rosenvold et al. 2003; Sterten et al. 2010). Macroglycogen content was significantly affected by pre-slaughter handling ($p = .004$) and muscle ($p < .001$), showing lower values on pig rough handled and in LT muscle. Moreover, significant interactions between pre-slaughter handling and muscle ($p = .036$; Figure 1(a)) and between muscle and breed ($p = .004$; Figure 1(b)) were observed for macroglycogen. In pigs subjected to rough handling, there was not difference in macroglycogen content between LT and SM muscles while in pigs handled gently the macroglycogen content was higher in SM than in LT ($p < .001$). Probably, the increased movement of pigs subjected to physical effort before slaughter led to a decreasing of the macroglycogen levels, even if it is degraded mainly during aerobic exercise (Essén-Gustavsson et al. 2005). The higher content of macroglycogen in SM with respect to LT in gently handled pigs probably reflected differences between muscles in their myofiber composition and metabolic properties (Ruusunen and Puolanne 2004). Moreover, this difference was related to the breed of pigs. As shown in Figure 1(b), the difference of content of macroglycogen between LD and SM was found to be significant in ILW ($p < .001$).

The effects of pre-slaughter handling, breed and their interaction on pH_{24}, colour, drip loss, cooking loss and shear force determined on LT are shown

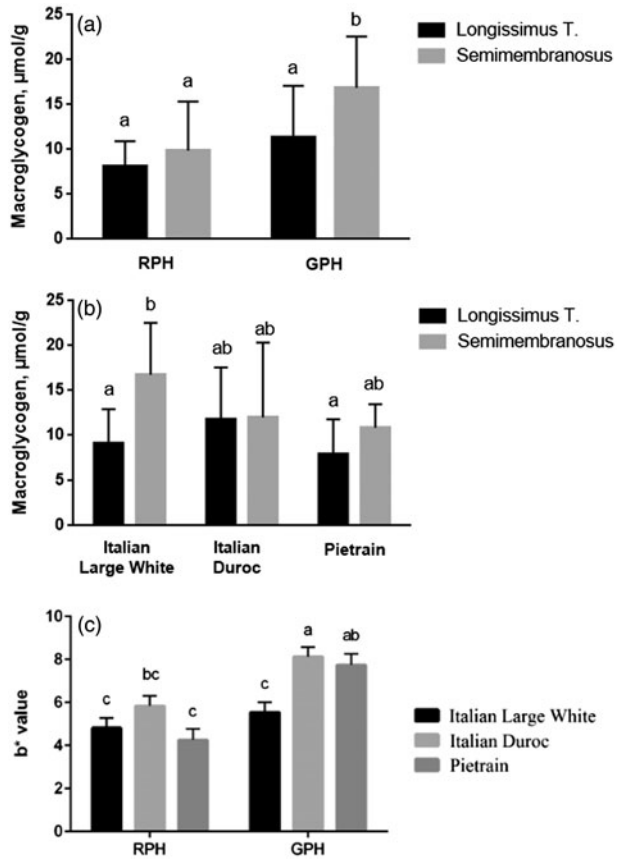

Figure 1. Effect of interaction between: (a) pre-slaughter handling and muscle on a macroglycogen; (b) muscle and breed on a macroglycogen; and (c) pre-slaughter handling and breed on b* 24 h value in *Longissimus thoracis*. [a,b,c]Different letters denote significant ($p < .05$) differences. RPH: rough pre-slaughter; GPH: gentle pre-slaughter.

in Table 2. Despite higher levels of lactate recorded in the roughly handled group, pH_{24} values were no significantly different between the two groups. This is probably related to the short intensity and duration of physical efforts before slaughter; comparable results were found in studies that evaluated similar pre-slaughter handlings (Rabaste et al. 2007; Dokmanović et al. 2014).

Table 2. Effect of pre-slaughter handling, breed and their interaction on pH_{24} colour, drip loss, cooking loss and shear force of *Longissimus thoracis*.

	Pre-slaughter handling, Pre-s.		Breed			*p* value			
Variable	Gentled	Rough	ILW	IDU	PI	Pre-s	Breed	Interaction	R-MSE
pH 24 h	5.51	5.50	5.55	5.51	5.47	ns	ns	ns	0.11
L* 24 h	54.31	53.27	51.10^{b}	53.61^{ab}	56.65^{a}	Ns	.001	ns	2.67
a* 24 h	9.17	8.45	8.64	9.16	8.63	Ns	ns	ns	1.93
b* 24 h	7.14^{a}	4.97^{b}	5.17^{b}	6.98^{a}	6.00^{ab}	.001	.003	.033^{c}	1.03
L* 72 h	55.75	56.84	53.45^{b}	56.41^{ab}	59.00^{a}	Ns	.017	ns	3.74
a* 72 h	4.15	3.40	3.64	4.15	3.53	Ns	ns	ns	1.67
b* 72 h	11.47	10.71	10.16^{b}	11.71^{a}	11.41^{a}	Ns	.004	ns	1.00
Drip loss, %	3.25	3.41	2.70	3.55	3.74	Ns	ns	ns	1.14
Cooking loss, %	18.41	16.97	18.80	17.92	16.35	Ns	ns	ns	3.57
Shear force, kgf	2.44	2.27	2.47	2.23	2.30	Ns	ns	ns	0.34

ns: not significant; ILW: Italian Large White; IDU: Italian Duroc; PI: Pietrain; R-MSE: root mean square error.
[a,b]Different letters in the same row denote significant ($p < .05$) differences.
[c]Interaction effect are shown in Figure 1.

Except for b* at 24 h, there were no significant effects of handling treatment on meat quality traits ($p > .05$); the lack of significant pre-slaughter handling effect on meat quality traits can be due to similar final pH levels in both the groups. Probably, the early acceleration of glycolysis due to the rough handling was not strong enough to have consequence on the ultimate quality of the meat.

In this study, breed was responsible for the main differences in pork colour. The L* value, measured 24 and 72 h after slaughter, was higher in PI than in ILW pigs ($p < .05$), the former showing a slightly paler LT muscle. Variations of colour coordinate between cosmopolitan pig breeds are reported in the literature according to the diet, slaughter weight, degree of marbling and productive systems (Rosenvold and Andersen 2003). Interaction between handling and breed was found to have a significant effect on the colour b* coordinate recorded at 24-h post-mortem on LT. As show in Figure 1, pre-slaughter handling influence on b* values varied according to the breed. The values of the coordinate in rough were lower than those in gentle handling group for ILW and PI. It seems that pigs subjected to more intense physical exertions tend to have lower b* values, showing a slightly yellow colour of meat.

In general, the stress experienced by the pig submitted to the rough handling had little influence on the investigated meat quality parameters, water holding capacity and tenderness. This result could be due to the short duration of the run that was related to the distance between the areas of lairage and stunning at the slaughterhouse.

Conclusions

Rough handling before slaughter was found to affect pH, lactate and macroglycogen contents at 45-min post-mortem without exert any influence on pro- and total glycogen and on the ultimate pH, water holding capacity and shear force. Breed affected only the instrumental meat colour measures. Irrespective of the different pre-slaughter handling, PI pigs showed slightly paler meat.

Disclosure statement

No potential conflict of interest was reported by the authors.

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